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(57) Abstract <p>The present invention is based, at least in part, on the discovery and validation of specific immunogenic peptide epitopes for various HLA class II DR molecules, representative of the worldwide population. Such peptides comprise an epitope, or analog thereof, which binds to an HLA class II molecule at an IC₅₀ of less than or equal to 1,000 nM.</p>			

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WO 99/61916

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IDENTIFICATION OF BROADLY REACTIVE DR. RESTRICTED EPITOPES

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of Provisional U.S.S.N. 60/087,192 filed 5/29/98. The application is also related to U.S.S.N. 09/009953, filed January 21, 1998, U.S.S.N. 60/036,713, filed January 23, 1997, and U.S.S.N. 60/037,432 filed February 7, 1997.

BACKGROUND OF THE INVENTION

Helper T lymphocytes (HTL) play several important functions in immunity to pathogens. Firstly, they provide help for induction of both CTL and antibody responses. By both direct contact and by secreting lymphokines such as IL2 and IL4, HTL promote and support the expansion and differentiation of T and B cell precursors into effector cells. In addition, HTL can also be effectors in their own right, an activity also mediated by direct cell contact and secretion of lymphokines, such as IFN γ and TNF α . HTL have been shown to have direct effector activity in case of tumors, as well as viral, bacterial, parasitic, and fungal infections.

HTL recognize a complex formed between class II MHC molecules and antigenic peptides, usually between 10 and 20 residues long, and with an average size of between 13 and 16 amino acids. Peptide-class II interactions have been analyzed in detail, both at the structural and functional level, and peptide motifs specific for various human and mouse class II molecules have been proposed.

In the last few years, epitope based vaccines have received considerable attention as a possible mean to develop novel prophylactic vaccines and immunotherapeutic strategies. Selection of appropriate T and B cell epitopes should allow to focus the immune system toward conserved epitopes of pathogens which are characterized by high sequence variability (such as HIV, HCV and Malaria).

In addition, focusing the immune response towards selected determinants could be of value in the case of various chronic viral diseases and cancer, where T cells directed against the immunodominant epitopes might have been inactivated while T cells specific for subdominant epitopes might have escaped T cell tolerance. The use of epitope

based vaccines also allows to avoid "suppressive" T cell determinants which induce TH₂ responses, in conditions where a TH₁ response is desirable, or vice versa.

Finally, epitope based vaccines also offer the opportunity to include in the vaccine construct epitopes that have been engineered to modulate their potency, either by increasing MHC binding affinity, or by alteration of its TCR contact residues, or both. Inclusion of completely synthetic non-natural or generically unrelated to the pathogen epitopes (such as TT derived "universal" epitopes), also represents a possible mean of modulating the HTL response toward a TH₁, or TH₂ phenotype.

Once appropriate epitope determinants have been defined, they can be assorted and delivered by various means, which include lipopeptides, viral delivery vectors, particles of viral or synthetic origin, naked or particle absorbed cDNA.

However, before appropriate epitopes can be defined, one major obstacle has to be overcome, namely the very high degree of polymorphism of the MHC molecules expressed in the human population. In fact, more than two hundred different types of HLA class I and class II molecules have already been identified. It has been demonstrated that in the case of HLA class I molecules, peptides capable of binding several different HLA class I molecules can be identified. Over 60% of the known HLA class I molecules can, in fact, be grouped in four broad HLA supertypes, characterized by similar peptide binding specificities (HLA supermotifs).

In the case of class II molecules, it is also known that peptides capable of binding multiple HLA types and of being immunogenic in the context of different HLA molecules do indeed exist. Specific immunogenic peptide have not been readily identified, particularly those reactive with a large number of allelic products.

The present invention addresses these and other needs.

SUMMARY OF THE INVENTION

The present invention is based, at least in part, on the discovery and validation of specific immunogenic peptide epitopes for various HLA class II DR molecules, representative of the worldwide population. Such peptides comprise an epitope, or analog thereof, which binds to an HLA class II molecule at an IC₅₀ of less than or equal to 1,000 nM. Epitopes of the invention have been identified in a variety of antigens including tumor associated antigens such as carcinoembryonic antigen (CEA), p53, MAGE-2, MAGE-3, or

Her2/neu; viral antigens such as those from HIV, HBV, or HCV; and parasites such as *Plasmodium falciparum*.

The HLA class II binding peptides of the invention may further comprise an epitope having an amino acid that is Y, F, W, L, I, V, or M at the first position from the N-terminus of the epitope and an amino acid of S, T, C, A, P, V, I, L, or M at the sixth position from the N-terminus of the epitope.

A peptide epitope of the invention, or a nucleic acid that encodes a peptide of the invention, may be used, *inter alia*, as a pharmaceutical composition to induce a helper T cell response in a patient by contacting a helper T cell with the epitope. One or more peptide epitopes of the invention may be included in such a composition. In a preferred embodiment, one or more epitopes is presented to a helper T cell by an antigen-presenting cell that has been pulsed with the peptide *ex vivo*.

Definitions

The term "peptide" is used interchangeably with "oligopeptide" in the present specification to designate a series of residues, typically L-amino acids, connected one to the other typically by peptide bonds between the alpha-amino and carbonyl groups of adjacent amino acids. The oligopeptides of the invention are typically less than about 50 residues in length and usually consist of between about 10 and about 30 residues, more usually between about 12 and 25, and often 15 and about 20 residues.

An "immunogenic peptide" is a peptide which comprises an allele-specific motif such that the peptide will bind an MHC molecule and induce an HTL response. Immunogenic peptides of the invention are capable of binding to an appropriate HLA molecule and inducing HTL response against the antigen from which the immunogenic peptide is derived.

With regard to a particular amino acid sequence, an "epitope" is a set of amino acid residues which is involved in recognition by a particular immunoglobulin, or in the context of T cells, those residues necessary for recognition by T cell receptor proteins and/or Major Histocompatibility Complex (MHC) receptors. In an immune system setting, *in vivo* or *in vitro*, an epitope is the collective features of a molecule, such as primary, secondary and tertiary peptide structure, and charge, that together form a site recognized by an immunoglobulin, T cell receptor or HLA molecule.

A "conserved residue" is a conserved amino acid occupying a particular position in a peptide motif typically one where the MHC structure may provide a contact point with the immunogenic peptide. One to three, typically two, conserved residues within a peptide of defined length defines a motif for an immunogenic peptide. These residues are typically in close contact with the peptide binding groove, with their side chains buried in specific pockets of the groove itself.

The term "motif" refers to the pattern of residues in a peptide of defined length, usually a peptide of from about 8 to about 13 amino acids for a class I HLA motif and from about 6 to about 25 amino acids for a class II HLA motif, which is recognized by a particular HLA molecule. Peptide motifs are typically different for each protein encoded by each human HLA allele and differ in the pattern of the primary and secondary anchor residues.

The term "supermotif" refers to motifs that, when present in an immunogenic peptide, allow the peptide to bind more than one HLA antigen. The supermotif preferably is recognized by at least one HLA allele having a wide distribution in the human population, preferably recognized by at least two alleles, more preferably recognized by at least three alleles, and most preferably recognized by more than three alleles.

A "primary anchor residue" is an amino acid at a specific position along a peptide sequence which is understood to provide a contact point between the immunogenic peptide and the HLA molecule. One to three primary anchor residues within a peptide of defined length generally defines a "motif" for an immunogenic peptide. These residues are understood to fit in close contact with peptide binding grooves of an HLA molecule, with their side chains buried in specific pockets of the binding grooves themselves. For example, analog peptides can be created by altering the presence or absence of particular residues in these primary anchor positions. Such analogs are used to modulate the binding affinity of a peptide comprising a particular motif or supermotif.

A "secondary anchor residue" is an amino acid at a position other than a primary anchor position in a peptide which may influence peptide binding. A secondary anchor residue occurs at a significantly higher frequency amongst bound peptides than would be expected by random distribution of amino acids at one position. The secondary anchor residues are said to occur at "secondary anchor positions." A secondary anchor residue can be identified as a residue which is present at a higher frequency among high or intermediate affinity binding peptides, or a residue otherwise associated with high or intermediate affinity

binding. For example, analog peptides can be created by altering the presence or absence of particular residues in these secondary anchor positions. Such analogs are used to finely modulate the binding affinity of a peptide comprising a particular motif or supermotif.

A "negative binding residue" is an amino acid which if present at certain positions (typically not primary anchor positions) of peptide epitope results in decreased binding affinity of the peptide for the peptide's corresponding HLA molecule.

Throughout this disclosure, results are expressed in terms of "IC₅₀'s." IC₅₀ is the concentration of peptide in a binding assay at which 50% inhibition of binding of a reference peptide is observed. Given the conditions in which the assays are run (*i.e.*, limiting HLA proteins and labeled peptide concentrations), these values approximate K_d values. It should be noted that IC₅₀ values can change, often dramatically, if the assay conditions are varied, and depending on the particular reagents used (*e.g.*, HLA preparation, *etc.*). For example, excessive concentrations of HLA molecules will increase the apparent measured IC₅₀ of a given ligand. Assays for determining binding are described in detail in PCT publications WO 94/20127 and WO 94/03205. Alternatively, binding is expressed relative to a reference peptide. As a particular assay becomes more, or less, sensitive, the IC₅₀'s of the peptides tested may change somewhat. However, the binding relative to the reference peptide will not significantly change. For example, in an assay run under conditions such that the IC₅₀ of the reference peptide increases 10-fold, the IC₅₀ values of the test peptides will also shift approximately 10-fold. Therefore, to avoid ambiguities, the assessment of whether a peptide is a good, intermediate, weak, or negative binder is generally based on its IC₅₀, relative to the IC₅₀ of a standard peptide.

As used herein, "high affinity" with respect to HLA class II molecules is defined as binding with an IC₅₀ or K_d of less than 100 nM. "Intermediate affinity" is binding with an IC₅₀ or K_d of between about 100 and about 1000 nM.

"Cross-reactive binding" indicates that a peptide is bound by more than one HLA molecule; a synonym is degenerate binding.

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state. Thus, the peptides of this invention do not contain materials normally associated with their *in situ* environment, *e.g.*, MHC I molecules on antigen presenting cells. Even where a protein has been isolated to a homogenous or dominant band, there are trace contaminants in the range of 5-10% of native protein which co-purify with the desired

protein. Isolated peptides of this invention do not contain such endogenous co-purified protein.

The term "residue" refers to an amino acid or amino acid mimetic incorporated in an oligopeptide by an amide bond or amide bond mimetic.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 presents a map of the positive or negative effect of each of the 20 naturally occurring amino acids on DR4w4 binding capacity when occupying a particular position, relative to the main P1-P6 anchors.

Figure 2A presents a map of the positive or negative effect of each of the 20 naturally occurring amino acids on DR1 binding capacity when occupying a particular position, relative to the main P1-P6 anchors.

Figure 2B presents a map of the positive or negative effect of each of the 20 naturally occurring amino acids on DR7 binding capacity when occupying a particular position, relative to the main P1-P6 anchors.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention relates to compositions and methods for preventing, treating or diagnosing a number of pathological states such as viral, fungal, bacterial and parasitic diseases and cancers. In particular, it provides novel peptides capable of binding selected major histocompatibility complex (MHC) class II molecules at an IC_{50} of less than or equal to 1000 nM and inducing an immune response.

Peptide binding to MHC molecules is determined by the allelic type of the MHC molecule and the amino acid sequence of the peptide. MHC class II-binding peptides usually contain within their sequence two conserved ("anchor") residues that interact with corresponding binding pockets in the MHC molecule. Specific combination of anchor residues (usually referred to as "MHC motifs") required for binding by several allelic forms of human MHC (HLA, histocompatibility leukocyte antigens) are described in International Applications WO 94/03205 and WO 94/20127. Definition of specific MHC motifs allows one to predict from the amino acid sequence of an individual protein, which peptides have the potential of being immunogenic for HTL. These applications describe methods for preparation and use of immunogenic peptides in the treatment of disease.

An affinity threshold strongly correlated with immunogenicity in the context of HLA class II DR molecules has been delineated as disclosed herein. In order to define a biologically significant threshold of DR binding affinity, a database of the binding affinities of 32 DR-restricted epitopes for their restricting element (*i.e.*, the HLA molecule that binds the motif) was compiled. In approximately half of the cases (15 of 32 epitopes), DR restriction was associated with high binding affinities, *i.e.* binding affinities of less than 100 nM. In the other half of the cases (16 of 32), DR restriction was associated with intermediate affinity (binding affinities in the 100-1000 nM range). In only one of 32 cases was DR restriction associated with an IC_{50} of 1000 nM or greater. Thus, 1000 nM, preferably 100 nM, can be defined as an affinity threshold associated with immunogenicity in the context of DR molecules.

The peptide epitopes described here can also be used in combination with peptide epitopes which induce a CTL response. See, also, WO 95/07077.

The peptide epitopes of the invention may also include analogs of the epitopes. Although the peptide epitopes may exhibit cross-reactive binding with multiple DR alleles, cross-reactivity is not always complete and in such cases procedures to further increase cross-reactivity of peptides can be useful; such procedures can also be used to modify other properties of the peptide epitopes. Having established the general rules that govern cross-reactivity of peptides for HLA alleles within a given motif or supermotif, modification (*i.e.*, analoging) of the structure of peptides of particular interest in order to achieve broader (or otherwise modified) HLA binding capacity can be performed. More specifically, peptides which exhibit the broadest cross-reactivity patterns, (both amongst the known T cell epitopes, as well as the more extended set of peptides that contain the appropriate supermotifs), can be produced in accordance with the teachings herein.

The strategy employed utilizes the motifs or supermotifs which correlate with binding to certain HLA molecules. The motifs or supermotifs are defined by having primary anchors, though secondary anchors can also be modified. Analog peptides can be created by substituting amino acids residues at primary anchor, secondary anchor, or at primary and secondary anchor positions. Generally, analogs are made for peptides that already bear a motif or supermotif. Preferred secondary anchor residues of supermotifs and motifs that have been defined for HLA class II binding peptides are shown in Table IX.

For a number of the motifs or supermotifs in accordance with the invention, residues are defined which are deleterious to binding to allele-specific HLA molecules or

members of HLA supertypes that bind to the respective motif or supermotif. Accordingly, removal of residues that are detrimental to binding can be performed in accordance with the present invention. For example, in the case of the A3 supertype, when all peptides that have such deleterious residues are removed from the population of analyzed peptides, the incidence of cross-reactivity increases from 22% to 37% (see, e.g., Sidney, J. *et al.*, *Hu. Immunol.* 45:79, 1996). Thus, one strategy to improve the cross-reactivity of peptides within a given supermotif is simply to delete one or more of the deleterious residues present within a peptide and substitute a small "neutral" residue such as Ala (that may not influence T cell recognition of the peptide). An enhanced likelihood of cross-reactivity is expected if, together with elimination of detrimental residues within a peptide, residues associated with high affinity binding to multiple alleles within a superfamily are inserted.

To ensure that changes in the native or original epitope recognized by T cells do not lead to a failure to elicit helper T cells that cross-react with the wild type peptides, the variant peptide may be used to immunize T cells *in vitro* from individuals of the appropriate HLA allele, and the cells' capacity to induce lysis of wild type peptide sensitized target cells is evaluated. In both class I and class II systems it will be desirable to use as targets, cells that have been either infected or transfected with the appropriate genes to establish whether endogenously produced antigen is also recognized by the relevant T cells.

Another embodiment of the invention to ensure adequate numbers of cross-reactive cellular binders is to create analogs of weak binding peptides. Class II peptides exhibiting binding affinities of above 1000 nM, and carrying an acceptable but suboptimal primary anchor residue at one or both positions can be "fixed" by substituting preferred anchor residues in accordance with the respective supertype. The analog peptides can then be tested for crossbinding activity.

Another embodiment for generating effective peptide analogs involves the substitution of residues that have an adverse impact on peptide stability or solubility in a liquid environment. This substitution may occur at any position of the peptide epitope. For example, a cysteine (C) can be substituted out in favor of α -amino butyric acid. Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity. Substituting α -amino butyric acid for C not only alleviates this problem, but actually improves binding and crossbinding capability in certain instances (Review: A. Sette *et al.*, In: *Persistent Viral Infections*, Eds. R. Ahmed and I. Chen, John Wiley & Sons, England, in press, 1998). Substitution of cysteine

with α -amino butyric acid may occur at any residue of a peptide epitope, i.e. at either anchor or non-anchor positions.

The DR-binding peptides of the present invention or nucleic acids encoding them can be administered to mammals, particularly humans, for prophylactic and/or therapeutic purposes. The DR peptide epitopes can be used to enhance immune responses against other immunogens administered with the peptides. For instance, CTL epitope/DR epitope mixtures may be used to treat and/or prevent viral infection and cancer. Alternatively, immunogens which induce antibody responses can be used. Examples of diseases which can be treated using the immunogenic mixtures of DR peptides and other immunogens include prostate cancer, hepatitis B, hepatitis C, AIDS, renal carcinoma, cervical carcinoma, lymphoma, CMV and condyloma acuminatum.

The DR-binding peptides or nucleic acids encoding them may also be used to treat a variety of conditions involving unwanted T cell reactivity. Examples of diseases which can be treated using DR-binding peptides include autoimmune diseases (e.g., rheumatoid arthritis, multiple sclerosis, and myasthenia gravis), allograft rejection, allergies (e.g., pollen allergies), Lyme disease, hepatitis, LCMV, post-streptococcal endocarditis, or glomerulonephritis, and food hypersensitivities.

In therapeutic applications, the immunogenic compositions or the DR-binding peptides or nucleic acids of the invention are administered to an individual already suffering from cancer, autoimmune disease, or infected with the virus of interest. Those in the incubation phase or the acute phase of the disease may be treated with the DR-binding peptides or immunogenic conjugates separately or in conjunction with other treatments, as appropriate.

In therapeutic applications, compositions comprising immunogenic compositions are administered to a patient in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, e.g., the peptide composition, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician.

Therapeutically effective amounts of the immunogenic compositions of the present invention are in the general range of immunogenically effective dosages described below. These doses may be followed by boosting dosages pursuant to a boosting regimen

over weeks to months depending upon the patient's response and condition by measuring specific HTL activity in the patient's blood.

It must be kept in mind that the compositions of the present invention may generally be employed in serious disease states, that is, life-threatening or potentially life-threatening situations. In such cases, in view of the minimization of extraneous substances and the relative nontoxic nature of the conjugates, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these compositions.

For prophylactic use, administration should be given to risk groups. For example, protection against malaria, hepatitis, or AIDS may be accomplished by prophylactically administering compositions of the invention, thereby increasing immune capacity. Therapeutic administration may begin at the first sign of disease or the detection or surgical removal of tumors or shortly after diagnosis in the case of acute infection. This is followed by boosting doses until at least symptoms are substantially abated and for a period thereafter. In chronic infection, loading doses followed by boosting doses may be required.

Treatment of an infected individual with the compositions of the invention may hasten resolution of the infection in acutely infected individuals. For those individuals susceptible (or predisposed) to developing chronic infection the compositions are particularly useful in methods for preventing the evolution from acute to chronic infection. Where the susceptible individuals are identified prior to or during infection, for instance, as described herein, the composition can be targeted to them, minimizing need for administration to a larger population.

The peptide mixtures or conjugates can also be used for the treatment of chronic infection and to stimulate the immune system to eliminate virus-infected cells in carriers. It is important to provide an amount of immuno-potentiating peptide in a formulation and mode of administration sufficient to effectively stimulate helper T cell response. Immunizing doses followed by boosting doses at established intervals, e.g., from one to four weeks, may be required, possibly for a prolonged period of time to effectively immunize an individual. In the case of chronic infection, administration should continue until at least clinical symptoms or laboratory tests indicate that the viral infection has been eliminated or substantially abated and for a period thereafter.

The pharmaceutical compositions for therapeutic or prophylactic treatment are intended for parenteral, topical, oral or local administration. Typically, the pharmaceutical compositions are administered parenterally, e.g., intravenously, intrathecally,

subcutaneously, intradermally, or intramuscularly. Because of the ease of administration, the vaccine compositions of the invention are particularly suitable for oral administration. Thus, the invention provides compositions for parenteral administration which comprise a solution of the peptides or conjugates dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, e.g., water, buffered water, 0.9% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

The concentration of DR and/or CTL stimulatory peptides of the invention in the pharmaceutical formulations can vary widely, i.e., from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

The peptides or conjugates of the invention may also be administered via liposomes, which serve to target the conjugates to a particular tissue, such as lymphoid tissue, or targeted selectively to infected cells, as well as increase the half-life of the peptide composition. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations the peptide to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to, e.g., a receptor prevalent among lymphoid cells, such as monoclonal antibodies which bind to the CD45 antigen, or with other therapeutic or immunogenic compositions. Thus, liposomes filled with a desired peptide or conjugate of the invention can be directed to the site of lymphoid cells, where the liposomes then deliver the selected therapeutic/immunogenic peptide compositions. Liposomes for use in the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, e.g., liposome size, acid lability and stability

of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka, *et al.*, *Ann. Rev. Biophys. Bioeng.* 9, 467 (1980), U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369, incorporated herein by reference.

For targeting to the immune cells, a ligand to be incorporated into the liposome can include, e.g., antibodies or fragments thereof specific for cell surface determinants of the desired immune system cells. A liposome suspension containing a peptide or conjugate may be administered intravenously, locally, topically, etc. in a dose which varies according to, inter alia, the manner of administration, the conjugate being delivered, and the stage of the disease being treated.

Alternatively, DNA or RNA encoding one or more DR peptides (and optionally, a polypeptide containing one or more CTL epitopes or antibody inducing epitopes) may be introduced into patients to obtain an immune response to the polypeptides which the nucleic acid encodes. This approach is described, for instance, in Wolff *et al.*, *Science* 247:1465 (1990) as well as U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; WO 98/04720; and in more detail below. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivacaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") delivery.

A preferred means of administering nucleic acids encoding the peptides of the invention uses minigene constructs encoding one or multiple epitopes of the invention. The use of multi-epitope minigenes is described below and in, e.g. An, L. and Whitton, J. L., *J. Virol.* 71:2292, 1997; Thomson, S. A. *et al.*, *J. Immunol.* 157:822, 1996; Whitton, J. L. *et al.*, *J. Virol.* 67:348, 1993; Hanke, R. *et al.*, *Vaccine* 16:426, 1998. For example, a multi-epitope DNA plasmid encoding nine dominant HLA-A*0201- and A11-restricted epitopes derived from the polymerase, envelope, and core proteins of HBV and HIV, the PADRE™ universal helper T cell (HTL) epitope, and an ER-translocating signal sequence was engineered. Immunization of HLA transgenic mice with this plasmid construct resulted in strong CTL induction responses against the nine epitopes tested, similar to those observed with a lipopeptide of known immunogenicity in humans, and significantly greater than immunization in oil-based adjuvants. Moreover, the immunogenicity of DNA-encoded epitopes *in vivo* correlated with the *in vitro* responses of specific CTL lines against target cells transfected with the DNA plasmid.

For example, to create a DNA sequence encoding the selected epitopes (minigene) for expression in human cells, the amino acid sequences of the epitopes may be reverse translated. A human codon usage table can be used to guide the codon choice for each amino acid. These epitope-encoding DNA sequences may be directly adjoined, so that when translated, a continuous polypeptide sequence is created. To optimize expression and/or immunogenicity, additional elements can be incorporated into the minigene design. Examples of amino acid sequences that could be reverse translated and included in the minigene sequence include: HLA class I epitopes, HLA class II epitopes, a ubiquitination signal sequence, a leader sequence, and/or an endoplasmic reticulum targeting signal. In addition, HLA presentation of CTL and HTL epitopes may be improved by including synthetic (*e.g.* poly-alanine) or naturally-occurring flanking sequences adjacent to the CTL or HTL epitopes.

The minigene sequence may be converted to DNA by assembling oligonucleotides that encode the plus and minus strands of the minigene. Overlapping oligonucleotides (30-100 bases long) may be synthesized, phosphorylated, purified and annealed under appropriate conditions using well known techniques. The ends of the oligonucleotides can be joined, for example, using T4 DNA ligase. This synthetic minigene, encoding the epitope polypeptide, can then be cloned into a desired expression vector.

Standard regulatory sequences well known to those of skill in the art are preferably included in the vector to ensure expression in the target cells. Several vector elements are desirable: a promoter with a down-stream cloning site for minigene insertion; a polyadenylation signal for efficient transcription termination; an *E. coli* origin of replication; and an *E. coli* selectable marker (*e.g.* ampicillin or kanamycin resistance). Numerous promoters can be used for this purpose, *e.g.*, the human cytomegalovirus (hCMV) promoter. *See, e.g.*, U.S. Patent Nos. 5,580,859 and 5,589,466 for other suitable promoter sequences.

Additional vector modifications may be desired to optimize minigene expression and immunogenicity. In some cases, introns are required for efficient gene expression, and one or more synthetic or naturally-occurring introns could be incorporated into the transcribed region of the minigene. The inclusion of mRNA stabilization sequences and sequences for replication in mammalian cells may also be considered for increasing minigene expression.

Once an expression vector is selected, the minigene is cloned into the polylinker region downstream of the promoter. This plasmid is transformed into an

appropriate *E. coli* strain, and DNA is prepared using standard techniques. The orientation and DNA sequence of the minigene, as well as all other elements included in the vector, are confirmed using restriction mapping and DNA sequence analysis. Bacterial cells harboring the correct plasmid can be stored as a master cell bank and a working cell bank.

In addition, immunostimulatory sequences (ISSs or CpGs) appear to play a role in the immunogenicity of DNA vaccines. These sequences may be included in the vector, outside the minigene coding sequence, if desired to enhance immunogenicity.

In some embodiments, a bi-cistronic expression vector which allows production of both the minigene-encoded epitopes and a second protein (included to enhance or decrease immunogenicity) can be used. Examples of proteins or polypeptides that could beneficially enhance the immune response if co-expressed include cytokines (e.g., IL-2, IL-12, GM-CSF), cytokine-inducing molecules (e.g., LeIF) or costimulatory molecules. Helper (HTL) epitopes can be joined to intracellular targeting signals and expressed separately from expressed CTL epitopes; this allows direction of the HTL epitopes to a cell compartment different than that of the CTL epitopes. If required, this could facilitate more efficient entry of HTL epitopes into the HLA class II pathway, thereby improving CTL induction. In contrast to HTL or CTL induction, specifically decreasing the immune response by co-expression of immunosuppressive molecules (e.g. TGF- β) may be beneficial in certain diseases).

Therapeutic quantities of plasmid DNA can be produced for example, by fermentation in *E. coli*, followed by purification. Aliquots from the working cell bank are used to inoculate growth medium, and grown to saturation in shaker flasks or a bioreactor according to well known techniques. Plasmid DNA can be purified using standard bioseparation technologies such as solid phase anion-exchange resins supplied by QIAGEN, Inc. (Valencia, California). If required, supercoiled DNA can be isolated from the open circular and linear forms using gel electrophoresis or other methods.

Purified plasmid DNA can be prepared for injection using a variety of formulations. The simplest of these is reconstitution of lyophilized DNA in sterile phosphate-buffer saline (PBS). This approach, known as "naked DNA," is currently being used for intramuscular (IM) administration in clinical trials. To maximize the immunotherapeutic effects of minigene DNA vaccines, an alternative method for formulating purified plasmid DNA may be desirable. A variety of methods have been described, and new techniques may become available. Cationic lipids can also be used in the

formulation (*see, e.g.*, as described by WO 93/24640; Mannino & Gould-Fogerite, *BioTechniques* 6(7): 682 (1988); U.S. Pat No. 5,279,833; WO 91/06309; and Felgner, *et al.*, *Proc. Nat'l Acad. Sci. USA* 84:7413 (1987). In addition, glycolipids, fusogenic liposomes, peptides and compounds referred to collectively as protective, interactive, non-condensing (PINC) could also be complexed to purified plasmid DNA to influence variables such as stability, intramuscular dispersion, or trafficking to specific organs or cell types.

In vitro assays can be used as functional assays for expression of HTL epitopes. For example, the plasmid DNA is introduced into a mammalian cell line that is a suitable presenter of HTL epitopes.. The transfection method used will be dependent on the final formulation. Electroporation can be used for "naked" DNA, whereas cationic lipids allow direct *in vitro* transfection. A plasmid expressing green fluorescent protein (GFP) can be co-transfected to allow enrichment of transfected cells using fluorescence activated cell sorting (FACS). The cells may then be assayed for the ability to elicit an HTL response using methods known in the art (*see, e.g.*, Alexander *et al.*, *Immunity* 1:751-761, 1994)

In vivo immunogenicity is a second approach for functional testing of minigene DNA formulations which include both CTL and HTL epitopes. Transgenic mice expressing appropriate human HLA proteins are immunized with the DNA product. The dose and route of administration are formulation dependent (*e.g.*, IM for DNA in PBS, IP for lipid-complexed DNA). Twenty-one days after immunization, splenocytes are harvested and restimulated for 1 week in the presence of peptides encoding each epitope being tested. For CTL effector cells, assays are conducted for cytolysis of peptide-loaded, chromium-51 labeled target cells using standard techniques. Lysis of target cells sensitized by HLA loading of peptides corresponding to minigene-encoded epitopes demonstrates DNA vaccine function for *in vivo* induction of CTLs.

Alternatively, the nucleic acids can be administered using ballistic delivery as described, for instance, in U.S. Patent No. 5,204,253. Using this technique, particles comprised solely of DNA are administered. In a further alternative embodiment, DNA can be adhered to particles, such as gold particles.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers

previously listed, and generally 10-95% of active ingredient, that is, one or more conjugates of the invention, and more preferably at a concentration of 25%-75%.

For aerosol administration, the peptides are preferably supplied in finely divided form along with a surfactant and propellant. Typical percentages of conjugates are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, as desired, as with, e.g., lecithin for intranasal delivery.

In another aspect the present invention is directed to vaccines which contain as an active ingredient an immunogenically effective amount of an immunogenic DR peptide or a CTL\DR peptide conjugate or nucleic acid encoding them as described herein. The conjugate(s) may be introduced into a host, including humans, linked to its own carrier or as a homopolymer or heteropolymer of active peptide units. Such a polymer has the advantage of increased immunological reaction and, where different peptides are used to make up the polymer, the additional ability to induce antibodies and/or CTLs that react with different antigenic determinants of the virus or tumor cells. Useful carriers are well known in the art, and include, e.g., thyroglobulin, albumins such as bovine serum albumin, tetanus toxoid, polyamino acids such as poly(lysine:glutamic acid), hepatitis B virus core protein, hepatitis B virus recombinant vaccine and the like. The vaccines can also contain a physiologically tolerable (acceptable) diluent such as water, phosphate buffered saline, or saline, and further typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are materials well known in the art. And, as mentioned above, CTL responses can be primed by conjugating peptides of the invention to lipids, such as P₃CSS. Upon immunization with a peptide composition as described herein, via injection, aerosol, oral, transdermal or other route, the immune system of the host responds to the vaccine by producing large amounts of CTLs and/or HTLs specific for the desired antigen, and the host becomes at least partially immune to later infection, or resistant to developing chronic infection.

Vaccine compositions containing the DR peptides of the invention are administered to a patient susceptible to or otherwise at risk of disease, such as viral infection or cancer in an amount that will elicit an immune response against the antigen and thus enhance the patient's own immune response capabilities.

A therapeutically effective amount and an amount used for vaccine of a peptide disclosed herein is defined to be an "immunogenically effective dose." In this use, the precise amounts again depend on the patient's state of health and weight, the mode of administration, the nature of the formulation, etc., but generally occur in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1000 µg and the higher value is about 10,000; 20,000; 30,000; or 50,000 µg. Dosage values for a human typically range from about 500 µg to about 50,000 µg per 70 kilogram patient.

In some instances it may be desirable to combine the peptide vaccines of the invention with vaccines which induce neutralizing antibody responses to the virus of interest, particularly to viral envelope antigens. For instance, PADRE peptides can be combined with hepatitis vaccines to increase potency or broaden population coverage. Suitable hepatitis vaccines that can be used in this manner include, Recombivax HB® (Merck) and Engerix-B (Smith-Kline).

For therapeutic or immunization purposes, the peptides of the invention can also be expressed by attenuated viral hosts, such as vaccinia or fowlpox. This approach involves the use of vaccinia virus as a vector to express nucleotide sequences that encode the peptides of the invention. Upon introduction into an acutely or chronically infected host or into a non-infected host, the recombinant vaccinia virus expresses the immunogenic peptide, and thereby elicits a host CTL response. Vaccinia vectors and methods useful in immunization protocols are described in, e.g., U.S. Patent No. 4,722,848, incorporated herein by reference. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover *et al.*, *Nature* 351, 456-460 (1991)) which is incorporated herein by reference. A wide variety of other vectors useful for therapeutic administration or immunization of the peptides of the invention, e.g., Salmonella typhi vectors and the like, will be apparent to those skilled in the art from the description herein.

The peptide epitopes of the invention may be administered to antigen presenting cells (APCs), preferably dendritic cells, *ex vivo*, as well. In a preferred embodiment, responses to a particular pathogen (infectious agent or tumor antigen) are induced by pulsing APCs with the peptide epitope and subsequently administering the pulsed

APC, wherein the cells then present the peptide *in vivo*. The pulsed APCs may be administered *in vivo* as described above for the peptides.

Peptides epitopes of the invention may also be used in conjunction with CTL epitopes to elicit CTL *ex vivo* as well. The resulting CTL can be used to treat infections or tumors. *Ex vivo* CTL responses to a particular pathogen are induced by incubating in tissue culture the patient's CTL precursor cells together with a source of antigen-presenting cells and the appropriate immunogenic peptide epitopes. After an appropriate incubation time *typically 1-4 weeks) in which the CTL precursor cells are activated and expanded into effector CTL, the cells are infused back into the patient, where they will destroy their specific target cell (an infected cell or a tumor cell).

The peptides of this invention may also be used to make monoclonal antibodies. Such antibodies may be useful as potential diagnostic or therapeutic agents.

The peptides may also find use as diagnostic reagents. For example, a peptide of the invention may be used to determine the susceptibility of a particular individual to a treatment regimen which employs the peptide or related peptides, and thus may be helpful in modifying an existing treatment protocol or in determining a prognosis for an affected individual. In addition, the peptides may also be used to predict which individuals will be at substantial risk for developing chronic infection.

Examples

Materials and Methods

Cells. The following Epstein-Barr virus (EBV) transformed homozygous cell lines were used as sources of human HLA class II molecules: LG2 [DRB1c0101 (DR1)1]; GM3107 [DRB50101 (DR2w2a)]; MAT (DRB10301 (DR3)1); PREISS [DRB10401 (DR4w4)1]; BIN40 [DRB10404 (DR4w14)1]; SWEIG [DRB11101 (DR5w11)]; PITOUT [DRB10701 (DR7)] (a); KT3 [DRB10405 (DR4w15)]; Herluf [DRB11201 (DR5w12)]; HO301 [DRB11302 (DR6w19)]; OLL [DRB10802 (DR8w2)]; and HTC9074 [DRB10901 (DR9)], supplied as a kind gift by Dr. Paul Harris, Columbia University]. In some instances, transfected fibroblasts were used: L466.1 [DRB11501 (DR2w2b)]; TR81.19 [DRB30101 (DR52a)]; and L257.6 [DRB40101 (DRw53)]. (Valli, *et al. J. Clin. Invest.* 91:616 (1993). Cells were maintained *in vitro* by culture in RPMI 1640 medium supplemented with 2mM L-glutamine [GIBCO, Grand Island, NY], 50µM 2-ME, and 10% heat-inactivated FCS [Irvine Scientific, Santa Ana, CA]. Cells were also supplemented with 100 µg/ml of

streptomycin and 100U/ml of penicillin [Irvine Scientific]. Large quantities of cells were grown in spinner cultures.

Cells were lysed at a concentration of 10^8 cells/ml in PBS containing 1% NP-40 [Fluka Biochemika, Buchs, Switzerland], 1mM PMSF [CalBioChem, La Jolla, CA], 5mM Na-orthovanadate, and 25mM iodoacetamide [Sigma Chemical, St. Louis, Mo]. The lysates were cleared of debris and nuclei by centrifugation at $10,000 \times g$ for 20 min.

Affinity purification of HLA-DR molecules. Class II molecules were purified by affinity chromatography as previously described (Sette, *et al. J. Immunol.* 142:35 (1989) and Gorga, *et al. J. Biol. Chem.* 262:16087 (1987)) using the mAb LB3.1 coupled to Sepharose 4B beads. Lysates were filtered through 0.8 and $0.4 \mu\text{m}$ filters and then passed over the anti-DR column, which were then washed with 15-column volumes of 10mM TRIS in 1% NP-40, PBS and 2-column volumes of PBS containing 0.4% n-octylglucoside. Finally, the DR was eluted with 50mM diethylamine in 0.15M NaCl containing 0.4% n-octylglucoside, pH 11.5. A 1/25 volume of 2.0M Tris, pH 6.8, was added to the eluate to reduce the pH to ~ 8.0 , and then concentrated by centrifugation in Centriprep 30 concentrators at 2000 rpm (Amicon, Beverly, MA).

Class II peptide-binding assays. A panel of 13 different specific DR-peptide assays were utilized in the present study. These assays were chosen as to be representative of the most common DR alleles. Table I lists for each DR antigen, the representative allelic product utilized, the cell line utilized as a source of DR, and the radiolabeled probe utilized in the assay. Purified human class II molecules [5 to 500 nM] were incubated with various unlabeled peptide inhibitors and 1-10 nM ^{125}I -radiolabeled probe peptides for 48h in PBS containing 5% DMSO in the presence of a protease inhibitor cocktail. The radiolabeled probes used were HA Y307-319 (DR1), Tetanus Toxoid[TT] 830-843 (DR2w2a, DR5w111, DR7, DR8w2, DR8w3, DR9), MBP Y85-100 (DR2w2b), TT1272-1284 (DR52a), MT 65 kD Y3-13 with Y7 substituted with F for DR3, a non-natural peptide with the sequence YARFQSQTTLKQKT (DR4w4, DR4w15, DRw53) (Valli, *et al. supra*), and for DR5w12, a naturally processed peptide eluted from the cell line C1R, EALIHQLINPYVLS (DR5w12) and 650.22 peptide, (TT 830-843 A @ S836 analog), for DR6w19.

Radiolabeled peptides were iodinated using the chloramine-T method. Peptide inhibitors were typically tested at concentrations ranging from 1201 $\mu\text{g/ml}$ to 1.2 ng/ml. The data were then plotted and the dose yielding 50% inhibition (IC_{50}) was measured. In appropriate stoichiometric conditions, the IC_{50} of an unlabeled test peptide to

the purified DR is a reasonable approximation of the affinity of interaction (K_d). Peptides were tested in two to four completely independent experiments. The final concentrations of protease inhibitors were: 1mM PMSF, 1.3nM 1.10 phenanthroline, 73 μ M pepstatin A, 8mM EDTA, and 200 μ M N alpha-p-tosyl-L-lysine chloromethyl ketone (TLCK) [All protease inhibitors from CalBioChem, La Jolla, CA]. Final detergent concentration in the incubation mixture was 0.05% Nonidet P-40. Assays were performed at pH 7.0 with the exception of DR3, which was performed at pH 4.5, and DRw53, which was performed at pH 5.0. The pH was adjusted as previously described (Sette, *et al. J. Immunol.* 148:844 (1992)).

Class II peptide complexes were separated from free peptide by gel filtration on TSK2000 columns (TosoHaas 16215, Montgomeryville, PA), and the fraction of bound peptide calculated as previously described (Sette, *et al.*, (1989) *supra*). In preliminary experiments, the DR prep was titrated in the presence of fixed amounts of radiolabeled peptides to determine the concentration of class II molecules necessary to bind 10-20% of the total radioactivity. All subsequent inhibition and direct binding assays were the performed using these class II concentrations.

DRB1 specificity of DR4w15, DR6w19, DR8w2, DR8w3, and DR9 assays.

Because the antibody used for purification is α -chain specific, β 1 molecules are not separated from β 3 (and/or β 4 and β 5) molecules. Development and validation of assays in regard with DR β chain specificity has been described in detail elsewhere for many of the DR alleles listed above (108). Herein we describe for the first time DR4w15, DR6w19, DR8w2, DR8w3, and DR9 assays. Experiments addressing the β chain specificity of these new assays are described in the present section.

DR4w15. The β 4 product DRw53 is co-expressed with DR4w15 and the determination of the specificity of the DR4w15 binding assay is complicated in that the same radiolabeled ligand is used for both the DR4w15 and DRw53 binding assays. Since typically β 1 chains are expressed at 5-10 fold higher levels than other β chains, and all binding assays are performed utilizing limiting DR amounts, it would be predicted that the dominant specificity detected in the assay would be DR4w15. To verify that this was indeed the case, the binding pattern of a panel of 58 different synthetic peptides in the putative DR4w15 specific assay with that obtained in a DRw53 specific assay (which uses a DRw53 fibroblast as the source of class II molecules). Two very distinct binding patterns were noted, and in

several instances, a peptide bound to one DR molecule with high affinity, and did not bind to the other (data not shown).

DR6w19. The DR6w19 assay utilizes as the source of class II molecules the EBV transformed homozygous cell line H0301, which co-expresses DRB30301 (DR52a). While the radiolabeled ligand used in the DR6w19 assay is different than that used for the DR52a assay, the ligand is related (i.e., is a single substitution analog) to a high affinity DR52a binder. As was done in the case of DR4w15, the specificity of the assay was investigated by analyzing the binding capacity of a panel of naturally occurring peptides for DR6w19 and DR52a. The two assays demonstrated completely different binding specificities. For example, in terms of relative binding, TT 1272-1284 binds 63-fold better in the DR52a assay than in the DR6w19 assay. Conversely, the Invariant chain peptide binds 189-fold better in the DR6w19 assay. In conclusion, these data demonstrated that the binding of the radiolabeled peptide 650.22 to purified class II MHC from the H0301 cell line is specific for DR6w19.

DR8w2 and DR8w3. The $\beta 1$ specificity of the DR8w2 and DR8w3 assays is obvious in that no $\beta 3$ (and/or $\beta 4$ and $\beta 5$) molecule is expressed.

DR9. The specificity of DR9 assay is inferred from previous studies which have shown that the TT 830-843 radiolabeled probe peptide does not bind to DRw53 molecules (Alexander, *et al.*, *Immunity* 1:751 (1994)).

Results

DR binding affinity of antigenic peptides recognized by DR restricted T cells

To define a threshold DR binding affinity, to be considered as biologically significant, we compiled the affinities of a panel of 32 reported instances of DR restriction of a given T cell epitope. In approximately half of the cases, DR restriction was associated with affinities of less than 100 nM, and in the other half of the instances, with IC50% in the 100-1000 nM range. Only in 1 out of 32 cases (3.1%) DR restriction was associated with IC50% of 1000 nM or greater. It was noted that this distribution of affinities differs from what was previously reported for HLA class I epitopes, where a vast majority of epitopes bound with IC50% of 50 nM or less (Sette, *et al.*, *Jl*, 1994). This relatively lower affinity of class II restricted epitope interactions might explain why activation of class II restricted T cells in general requires more antigen relative to class I restricted T cells.

In conclusion, this analysis suggested that 1000 nM may be defined as an affinity threshold associated with immunogenicity in the context of DR molecules, and for this reason a suitable target for our studies.

P1 and P6 anchors are necessary but not sufficient for DRB10401 binding

Several independent studies have pointed to a crucial role in DRB10401 binding of a large aromatic or hydrophobic residue in position 1, near the N-terminus of the peptide and of a 9-residue core region (residues 1 through 9). In addition, an important role has been demonstrated for the residue in position six (P6) of this 9-residues core region. Short and/or hydrophobic residues were in general preferred in this position (O'Sullivan, *et al.*, JI 147:2663, 1991; Sette, *et al.*, JI 151:3163, 1993; Hammer, *et al.*, Cell 74:197, 1993 and Marshall, *et al.*, JI 154:5927, 1995).

In the present set of experiments, a library of 384 peptides was analyzed for DRB10401 binding capacity and screened for the presence of the P1-P6 motif (that is, F, W, Y, L, I, V or M in P1 and S, T, C, A, P, V, I, L or M in P6, at least 9 residues apart from the peptide C-terminus. This set of 384 peptides contained a total of 80 DR4w4 binders (specifically 27 good binders [IC₅₀ of 100 nM or less], and 53 intermediate binders [IC₅₀ of the 100-1000 range]. Seventy-seven out of the 80 DR4w4 binders (96%) carried the P1-P6 motif. However, it should be noted that most non-DR4w4 binding peptides also contained the P1-P6 motif. Of 384 peptides included in our database, only 125 were "P1-P6 negative." Only three of them (6%) bound appreciably to purified DR4w4 as opposed to 77/259 (30%) of the "P1-P6 positive" peptides. Therefore, these results demonstrate that presence of suitable P1 and P6 anchors are necessary but not sufficient for DRB10401 binding.

A detailed map of DRB10401 peptide interactions

Next, for each P1-P6 aligned core region, in analogy with what the strategy previously utilized to detail peptide class I interactions the average binding affinity of peptides carrying a particular residue, relative to the remainder of the group, were calculated for each position. Following this method a table of average relative binding (ARB) values was compiled. This table also represents a map of the positive or negative effect of each of the 20 naturally occurring amino acids on DRB10401 binding capacity when occupying a particular position, relative to the main P1-P6 anchors (Figure 1).

Variations in ARB values greater than four fold (ARB ³ 4 or £ 0.25) were arbitrarily considered significant and indicative of secondary effects of a given residue on

DR-peptide interactions. Most secondary effects were associated with positions 4, 7, and 9. These positions correspond to secondary anchors engaging shallow pockets on the DR molecule. In addition, significant secondary effects were detected for M in position 3 (ARB = 12.8) T in position 3 (ARB = 4.34) and I in position 5 (ARB = 4.4).

Development of a DRB10401 specific algorithm

Next, the ARB table was utilized to develop a DRB10401 specific algorithm. In order to predict 0401 binding propensity, each aligned P1-P6 sequence was scored by multiplying, for each position, the ARB value of the appropriate amino acid. According to this procedure, a numerical "algorithm score" was derived. If multiple P1-P6 alignments were possible, binding scores were calculated for each one and the best score was selected. The efficacy of this method in predicting 0401 binding capacity is shown in Table IIa.

Considering only peptides with algorithm scores above -17.00 narrowed the set of predicted peptides to 156. This set still contained 72 out of 80 (90%) of the total high or intermediate DR binders. Raising the cut-off to an algorithm score of -16.44 or higher still allowed identification of 60 out of 80 (75%) of the DR4w4 binding peptides. Of the whole 107 peptide set, twenty-five of them were either good or intermediate binders. In other words, as expected, increasing the algorithm score stringency predicted a smaller fraction of the total binders present in the set, but at the same time less false positive peptides were identified.

Blind test of the predictive power of the DRB10401 specific algorithm

To verify that the predictive capacity of our algorithm was not merely a reflection of having utilized the same data set to test and define the algorithm itself, we further examined its efficacy in a blind prediction test. For this scope we utilized data from an independent set of 50 peptides, whose binding affinities were known, but that had not been utilized in the derivation of the algorithm. As shown in Table IIb, the algorithm was effective in predicting DR4w4 binding capacity of this independent peptide set. The algorithm score of -17.00 identified a total 18 peptides. This set contained 3/3 (100%) of all good binders, and 8/11 (70%) of all intermediate binders in the entire test set of 50 peptides. Increasing the cut-off value to -16.44, identified a set of nine peptides. Seven of them (78%) were either good or intermediate binders. This set contained 7 out of 14 (50%) of the

binders contained in the blind prediction peptide set. In conclusion, these data supports the validity of the DR4w4 specific algorithm described above.

Detailed maps of DRB10401, DRB10101, and DRB10701 peptide binding specificities

Next, we analyzed the binding to purified DR1 and DR7 molecules for the same set of 384 peptides utilized to define the DR4w4 algorithm. It was found that this set contained 120 and 59 binders for the DR1 and DR7 alleles, respectively. A total of 158 peptides were capable of binding either DR1, DR4w4 or DR7. A large fraction of them (73/158; 46%) were also degenerate binders, which bound two or more of the three alleles thus far considered. Furthermore, we also found that more than 90% of the DR1 or DR7 good and intermediate binders carried the P1-P6 motif. Most importantly, 72 out of 73 (99%) degenerate DR binders carried this motif (data not shown). In conclusion, this analysis suggests that P1-P6 based algorithms might be utilized to effectively predict degenerate DR binders.

In analogy with what was described above for DR4w4 molecules, specific algorithms were designed for the DR1 and DR7 alleles. Figures 2A and 2B detail the allele specific maps defined according to this method.

As in the case of DRB10401, most secondary effects were concentrated in positions 4, 7 and 9. Position 4 was especially prominent in the case of DR1, while position 7 was the most prominent secondary anchor for DR7. Specific algorithms were developed based on these maps, and it was found that the cut-off values necessary to predict 75% or 90% of the binders were -19.32 and -20.28 for DR1, and 20.91 and -21.63 for DR7, respectively. Depending on the particular allele or cut off value selected, 40 to 60% of the predicted peptides were in fact good or intermediate binders (data not shown).

Development of a DR1-4-7 combined algorithm

Finally, we examined whether a combined algorithm would allow to predict degenerate binders. For this purpose, the sequences of the 384 peptides in our database were simultaneously screened with the three (DR1, 4w4, and 7) specific algorithms. It was found that an even 100 peptides were predicted (using the 75% cut off) to bind either two or three of the alleles considered. This set contained 59 out of 73 (81%) of the peptides which were in fact capable of degenerate 1-4-7 binding (defined as the capacity to bind to more than one of the DR1, 4w4 or 7 alleles) (Table III).

Definition of a target set of DR specificities, representative of the world population

The data presented in the preceding sections illustrates how peptides capable of binding multiple DR alleles can be identified by the use of a combined "1-4-7" algorithm. Next, we wished to examine whether the peptides exhibiting degenerate 1-4-7 binding behavior would also bind other common DR types as well. As a first step in our experimental strategy, we sought to define a set of target DR types representative of a large (~80%) fraction of the world population, irrespective of the ethnic population of origin. For this purpose, seven additional DR antigens were considered. For each one of the DR antigens considered in this study, (including DR1, 4 and 7), the estimated frequency in various ethnicities, according to the most recent HLA workshop (11th, 1991) is shown in Table IVa, together with the main subtypes thus far identified.

For the purpose of measuring peptide binding affinity to the various DR molecules, one representative subtype for each DR antigen was chosen (Table I). It should be noted that for most antigens, either one subtype is by far the most abundant, or alternatively a significant degree of similarity in the binding pattern displayed by the different, most abundant subtypes of each DR antigen is likely to exist (see comments column of Table IVb). One exception to this general trend is represented by the DR4 antigen, for which significant differences in peptide specificity between the 0401 and 0405 have been reported. Since both alleles are quite frequent (in Caucasians and Orientals, respectively) we included both DR 0401 and 0405 in the set of representative DR binding assays.

Our set of representative assays is mostly focused on allelic products of the gene, because these molecules appear to be the most abundantly expressed, serve as the dominant restricting element of most human class III responses analyzed thus far, and accurate methods for serologic and DNA typing most readily available. However, we have also considered in our analysis assays representative of DRB3/4/5 molecules (Table IVc). These molecules serve as a functional restriction element, and their peptide binding specificity has been previously shown to have certain similarities to the specificity of several common DR β , allelic products.

A general strategy for prediction of DR-degenerate binders.

To test whether the 1-4-7 combined algorithm would also predict degenerate binding to other common DR types, we measured the capacity of three different groups of synthetic peptides to bind the panel of purified HLA DR molecules. The three different peptide sets were: A) 36 peptides which did not score positive in the combined 1-4-7 algorithm (non-predictions), B) 36 peptides which did score positive for the 1-4-7 algorithm, at the 75% cut off level, but had been found upon actual testing not to be degenerate 1-4-7 binders ("wrong" predictions), and C) 29 peptides which scored positive in the 1-4-7 algorithm, and also proved upon experimental testing, to be actual 1-4-7 degenerate binders (correct predictions). The results of this analysis are shown in Table V.

Within the set of "non-predictions" peptides (Table Va) only 3 out of 34 (9%) bound at least two of the DR1, 4w4 or 7 molecules. Interestingly, 2 (1136.04 and 1136.29) out of 3 of these peptides were also rather crossreactive, and bound additional DR types (DR2w2 β 2, DR4w15, 5w11 and 8w2 in the case of 1136.04, and 2w2 β 2, 4w15, 9 and 5w12 in the case of 1136.29). Peptides from the "wrong predictions" peptide set (Table V5), by definition bound at the most only one of the DR1, 4w4 or DR7 molecules, and were also poorly degenerate or other DR types with only two peptides (1136.22 and 1188.35) binding a total of three DR molecules. Within this peptide set, no peptide bound four or more of the DR molecules tested (data not shown).

These results are contrasted by data obtained with the peptide set corresponding to peptides which were first predicted by the use of the combined 1, 4, 7 algorithm, and then experimentally found to be degenerate DR1-4-7 binding. Fourteen out of 29 peptides tested (48%) bound a total of five or more alleles. Four of them were remarkably degenerate (1188.16, 1188.32, 1188.34 and F107.09) and bound a total of nine out of the 11 DR molecules tested. In conclusion, these results suggest that a strategy based on the sequential use of a combined DR1, 4, 7 algorithm and quantitative DR1, 4, 7 binding assays can be utilized to identify broadly crossreactive DR binding peptides.

Definition of the HLA-DR 1-4-7 supertype

The data presented above also suggested that several common DR types are characterized by largely overlapping peptide binding repertoires. When this issue was analyzed in more detail, by analyzing the binding pattern of the thirty-two peptides from Table Va and b which were actual DR1-4-7 degenerate binders. Thirty-one of them (97%) bound DR1, 22 (69%) DR4w4 and 21 (66%) DR7. These files are contrasted with the low

percentages of binding observed amongst the remainder non-degenerate binding peptides (17/67 (25%), 8/67 (12%) and 7/67 (10%), for DR1, 4w4 and 7, respectively) (Table VII).

Interestingly, a large fraction of the 1-4-7 degenerate binders also bound certain other common DR types. Sixteen (50%) bound DR2w2a, 18 (56%) DR6w19, 18 (56%) DR2w2b and 20 (62%) DR9. In all cases, the frequency of binding in the non-1-4-7 degenerate peptide set was much lower (Table VIII).

Significant, albeit lower, frequencies of cross reactivity were noted also for DR4w15, DR5w11, and DR8w2 (in the 28 to 37% range). Finally, negligible levels of cross reactivity were observed in the case of DR3 and 5w12 and DR53. Further studies will address whether either of these two group of molecules (DR4w15, 5w11, and 8w2 on one hand, and DR3, DR53 and 5w12 on the other) might belong to different DR supertypes.

In conclusion, these data demonstrates that a large set of DR molecules encompassing DR1, 4w4, 2w2a, 2w2b, 7, 9 and 6w19 is characterized by largely overlapping peptide binding repertoires.

Discussion

In the present report we have analyzed the peptide binding specificity of a set of 13 different DR molecules, representative of DR types common among the worldwide population. Detailed maps of secondary anchors and secondary interactions have been derived for three of them (DR4w4, DR1 and DR7). Furthermore, we demonstrated that a set of at least seven different DR types share overlapping peptide binding repertoires; and consequently that broadly degenerate HLA DR binding peptides are a relatively common occurrence. This study also describes computerized procedures which should greatly assist in the task of identification of such degenerate peptides.

We would like to discuss the data in the context of our current understanding of peptide-class II interactions, as well as in the context of the recently described class I supermotifs. Finally, the potential implications of broadly degenerate class II epitopes for epitope based vaccine design should also be considered.

Firstly, our studies illustrate how the vast majority of the peptides binding with good affinity to DR4w4, DR1, DR7 and most of the other DR types analyzed in the current study (data not shown), are all characterized by a P1-P6 motif consistent with the one originally proposed by O'Sullivan, *et al.* Crystallographic analysis of DR1-peptide complexes revealed that the residues occupying these positions engage two complementary

pockets on the DR1 molecule, with the P1 position corresponding to the most crucial anchor residue and the deepest hydrophobic pocket. Our analysis also illustrates how other "secondary anchor" positions drastically influence in an allele-specific manner peptide binding capacity. Position 4 was found to be particularly crucial for DR1 binding, position 9 for DR4w4, and position 7 for DR7. These data are consistent with previous results which originally described such allele-specific anchors, and with crystallographic data which illustrates how these residues engage shallow pockets on the DR molecule.

Secondly, our studies illustrate how an approach based on alignment and calculation of average relative binding values of large peptide libraries allows definition of quantitative algorithms to predict binding capacity. The present study extends those observations to two other common HLA-DR types, and also illustrates how the combined use of the 1-4-7 algorithms can be of aid in identifying broadly degenerate DR binding peptides.

The data presented herein suggest that a group of common DR alleles, including at least DR1, DR2w2a, DR2w2b, DR4w4, DR6w19, DR7 and DR9 share a largely overlapping peptide repertoire. Degenerate peptide binding to multiple DR alleles, and recognition of the same epitope in the context of multiple DR types was originally described by Lanzavecchia, Sinigaglia's and Rothbard's groups. The present study provides a classification of alleles belonging to a main HLA-DR supertype (DR1-4-7-like) which includes DR1, DR2w2a, DR2w2b, DR4w4, DR7, DR9, DR6w19. On the basis of the data presented herein, at least two additional groups of alleles exist. The first group encodes for molecules with significant, albeit much reduced overlap with the 1-4-7-like supertype (DR4w15, 8w2, 5w11). The second group of alleles (5w12, 3w17, and w53) clearly has little repertoire association with the 1-4-7 supertype. In this context it is interesting to note that Hammer, *et al.* noted that good DR5w11 binding peptides are frequently characterized by positively charged P6 anchor (which would be poorly compatible) with the herein proposed 1-4-7 supermotif. It is also interesting to note that Sidney, *et al.* proposed that DR3w17 binds a set of peptides largely distinct from those bound by other common DR types. Future studies will have to determine whether any of the molecules listed above can be grouped in additional DR superotypes. Our group is currently investigating whether analysis of polymorphic residues lining the peptide binding pockets of DR can be utilized to aid in the classification and prediction of HLA DR superotypes.

We would like to comment on similarities and differences between the HLA DR supertype described herein and the recently described HLA class I supermotifs. Class I supermotifs are clear-cut and, as a rule, non-overlapping. Four of them have been described all approximately equally frequent amongst the worldwide population. By contrast, the repertoire defining the HLA DR supertype herein described is not clear-cut and overlaps, at least in part, with the repertoire of other alleles. It also appears that on the basis of the data presented in Tables I and IV, even if other DR supertypes exist, the DR1-4-7 is going to be by far the most abundantly represented worldwide.

Finally, we would like to point out the possible relevance of these data in terms of development of epitope based vaccines. Class II restricted HTL have been implicated in protection from, and termination of many important diseases. Inclusion of well defined class II epitopes in prophylactic or therapeutic vaccines may allow the immune response to focus towards conserved or subdominant epitopes, and avoid suppressive determinants. Based on the data presented herein (Table IV), the DR1-4-7 supertype would allow coverage in the 50 to 80% range, depending on the ethnicities considered. It is thus possible that broad and not ethnically biased population coverage could be achieved by considering a very limited number of peptide binding specificities.

Based on the results present above, the sequences of various antigens of interest were scanned for the presence of the DR 1-4-7 motifs. Peptides identified using this approach are broadly cross reactive, class II restricted T cell epitopes. Table VIII presents a listing of such peptides derived from various antigens and includes representative epitopes that bind one or more DR alleles at an IC_{50} of 1000 nM or less. The information in Table VIII includes the antigen from which the peptide was derived, and binding data expressed as IC_{50} values for the designated DR alleles as shown.

The above examples are provided to illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art. All publications, patents, and patent applications cited herein are hereby incorporated by reference for all purposes.

Table I

HLA-DR binding assays utilized in the present study.

Antigen	Allele	Alias	Representative Assay			Comments
			Cell Line	Radio-labeled Probe	Ref.	
DR1	DRB1*0101	(DR1)	LC2	HA Y307-319 ¹⁾	(8)	01 is the most prevalent DR1 allele.
DR2	DRB1*1501	(DR2w2b)	L466.1	MDP 88-102Y ²⁾	(8)	0101 is the most prevalent DR2 allele.
DR3	DRB1*0301	(DR3w17)	MAT	MT 65KD Y3-13 analog ³⁾	(8)	01 is the most prevalent DR3 allele in most major populations. 01 and 02 are split fairly evenly in NA Blacks.
DR4	DRB1*0401	(DR4w4)	Prelis	Non-natural peptide YAR ^{a)}	(8)	01 is the most prevalent DR4 allele.
	DRB1*0405	(DR4w15)	KT3	Non-natural peptide YAR	This paper	05 is the most prevalent DR4 allele in the Orient.
DR7	DRB1*0701	(DR7)	Pibout	TT 830-843 ^{b)}	(8)	01/02 vary at 1 pos., which is outside the binding groove.
DR8	DRB1*0802	(DR8w2)	OLL	TT 830-843	This paper	02 is dominant in most major population groups. 02 and 03 have nearly identical binding specificities (J. Sidney and A. Sette, unpublished observations).
DR9	DRB1*0901	(DR9)	9074 (H1D)	TT 830-843	This paper	DR9 splits are products of a silent mutation.
DR11	DRB1*1101	(DR5w11)	Sweig	TT 830-843	(8)	01 is the most prevalent DR11 allele, by far.
DR12	DRB1*1201	(DR5w12)	Heiluf	CIR derived peptide ^{a)}	(9)	01/02 are evenly distributed. These alleles differ at pos. 67, which does not appear strongly influence peptide binding.
DR13	DRB1*1302	(DR6w19)	HC301	650.22 (TT 830-843 analog) ^{b)}	(10)	02 is slightly more prevalent overall than 01. These alleles vary at pos. 86/critical in determining the P1 anchor specificity.
DR51	DRB5*0101	(DR2w2a)	GM3107	TT 830-843 ^{b)}	(8)	0101 is the most prevalent split.
DR53	DRB4*0101	(DR4, DR7, DR9)	L257.6	Non-natural peptide YAR ^{a)}	(8)	0101 is essentially the only allele.

1) YTKVYKQNTLKLAT
2) VVLPFSNVITPTPT
3) YKTIATDELAKE
4) EALFQLQNTPTVLS
5) QYIKANALKEPTE
6) Vail et al., J. Clin. Invest. 91:416, 1993.

Table II

An algorithm to predict DRB1*0401 binding capacity.
a) Original peptide set.

Selection Criteria	No. of peptides (Binding nM)			Total
	High ≤100	Inter. 100-1000	Non >1000	
None	27	53	304	384
P1-P6	27	50	182	259
-17.00 ¹⁾	27	45	84	156
-16.44 ²⁾	25	35	47	107

1) Algorithm score which predicts 90% of all binders.

2) Algorithm score which predicts 75% of all binders.

Table II

b) Blind test of the predictive power of the DRB1*0401 algorithm.

Selection Criteria	No. of peptides (Binding nM)			Total
	High ≤100	Inter. 100-1000	Non >1000	
None	3	11	36	50
P1-P6	3	9	28	40
-17.00	3	8	7	18
-16.44	3	4	2	9

Table III

A combined "1-4-7" algorithm.

Selection Criteria	Degenerate Binders ¹⁾	Percent of Total Degenerate Binders
None	73/384	100%
P1-P6	72/259	99%
Combined Algorithms (90% Cutoff Value)	67/147	92%
Combined Algorithms (75% Cutoff Value)	59/100	81%

1) Degenerate binders are defined as peptides binding at least two out of the three DR1, 4w4, and 7 molecules with an IC50 of 1 μ M or less.

Table IV

Phenotypic frequencies of 10 prevalent HLA-DR antigens							
Antigen	Alleles	Phenotypic Frequencies					Avg.
		Cauc.	Blk.	Jpn.	Chn.	Hisp.	
DR1	DRB1*0101-03	18.5	8.4	10.7	4.5	10.1	10.4
DR2	DRB1*1501-03	19.9	14.8	30.9	22.0	15.0	20.5
DR3	DRB1*0301-2	17.7	19.5	0.4	7.3	14.4	11.9
DR4	DRB1*0401-12	23.6	6.1	40.4	21.9	29.8	24.4
DR7	DRB1*0701-02	26.2	11.1	1.0	15.0	16.6	14.0
DR8	DRB1*0801-5	5.5	10.9	25.0	10.7	23.3	15.1
DR9	DRB1*09011,09012	3.6	4.7	24.5	19.9	6.7	11.9
DR11	DRB1*1101-05	17.0	18.0	4.9	19.4	18.1	15.5
DR12	DRB1*1201-02	2.8	5.5	13.1	17.6	5.7	8.9
DR13	DRB1*1301-06	21.7	16.5	14.6	12.2	10.5	15.1
Total		97.0	83.9	98.8	95.5	95.6	94.7

Table V
A) Non Predictions.

Blinding Capacity													
P-allele	DRI,4,7			Other Alleles								Total Alleles Bound	
	DR1	DR1*4	DR7	DR2*2b	DR2*2a	DR3	DR1*15	DR5*11	DR6*19	DR8*2	DR9		DR5*12
1136.29	32	437	138	13	468	-	715	6250	-	2970	183	1000	7
1136.04	24	20	333	1264	741	-	543	69	-	55	2885	-	6
1136.19	781	1915	1923	86	1250	-	415	183	1667	5052	3115	-	4
1136.10	-	-	508	-	702	-	350	445	-	1581	4167	9091	4
1136.02.01a	006	-	-	2844	16	-	1086	126	8750	306	-	9927	3
1136.33	116	-	-	2659	1667	-	563	-	-	-	2419	1364	3
1136.57	-	7001	356	3957	1667	-	1689	-	-	7313	3947	3571	2
1136.03	79	8654	2003	213	1250	-	-	690	8750	-	-	-	2
1136.06	1923	1364	-	313	4977	-	-	-	3182	-	-	-	2
1136.23	962	-	-	262	-	2727	626	6250	-	1776	-	-	2
1136.32	37	-	-	1717	1739	-	7600	1835	8750	3161	-	476	2
1136.33	52	-	-	8273	6250	-	6552	4000	-	6344	-	-	2
1136.41.01	576	780	-	-	419	-	-	396	-	2970	3000	-	2
1136.02.01a	-	-	-	-	769	-	-	9514	8750	-	-	-	1
1136.42	-	1875	-	-	-	-	-	-	761	-	-	2727	1
1136.54	8333	-	-	-	-	-	-	1980	-	1235	2614	316	1
1136.07.01b	1190	-	4650	1542	2857	-	-	-	-	-	2027	-	1
1136.08	-	492	-	-	-	-	-	-	-	-	-	-	1
1136.09	-	9275	3788	73	-	-	-	-	2917	-	-	3846	1
1136.25	1163	-	6250	28	3816	-	-	-	-	-	5000	-	1
1136.34	4545	545	3117	-	-	-	-	-	-	-	32931	-	1
1136.36	204	-	-	5688	-	-	-	-	-	-	-	-	1
1136.44	-	235	-	-	-	-	-	-	-	-	-	-	1
1136.69	-	-	-	-	-	-	-	-	5000	-	-	-	1
1136.40	-	-	-	-	-	-	1267	-	54	-	5769	-	1
1136.50	-	-	-	-	-	-	-	-	7000	-	-	-	0
1136.56	-	4500	-	-	-	-	-	-	-	-	-	-	0
1136.57	-	8654	-	6500	-	-	3918	7113	-	5506	-	-	0
1136.61	-	-	-	-	-	-	5758	1636	3500	3104	4688	-	0
1136.66	-	-	-	-	-	-	-	-	-	-	7779	-	0
1136.64	-	-	-	-	-	-	-	-	-	-	-	-	0
1136.68	-	-	-	-	-	-	-	-	-	-	-	-	0
1136.70	-	-	-	-	-	-	-	3784	-	-	-	-	0
1136.72	-	-	-	-	-	-	-	-	-	-	-	-	0
1136.03.01a	-	-	-	-	1903	-	-	7882	-	-	-	-	0

- Indicates binding affinity $\geq 10,000$ nM.

2 ml of 34 (5.9%) degenerate on 5 or more DR types.

Table V

D) Correct Predictions.

b) Correct Predictions.

Binding Capacity (IC50% nM)

Other Alleles

DR1,4,7

Peptide	DR1	DR1*4	DR7	DR3*2b	DR3*2c	DR3	DR1*15	DR3*11	DR3*19	DR3*2	DR3	DR3*12	Total Allotopes Bound
1188.16	3.7	7.1	14	1251	23	—	47	30	428	46	28	—	9
1189.32	3.1	41	167	—	29	—	1402	11	2.1	19	126	851	9
1188.31	14	12	66	370	110	1332	959	2703	3.7	68	19	497	9
F107.09	4.1	14	39	5028	206	—	314	963	469	305	29	—	9
27.412	14	282	138	—	323	—	—	31	20	53	590	2495	8
1188.45	26	9.0	57	260	123	757	1057	2532	3.9	28	16	—	8
1136.16	1.5	214	46	1635	34	—	741	3571	1296	488	68	3409	7
1136.21	2.2	31	52	2844	42	—	270	1212	259	1420	132	—	7
1136.11	0.89	99	9615	603	261	—	86	315	—	579	1974	—	7
27.392	41	449	33	310	2499	—	1668	1203	9.8	883	62	—	6
27.417	56	—	425	210	251	—	—	471	33	2177	859	3713	6
1136.38	70	122	2404	258	711	—	133	4000	1812	—	862	—	4
27.388	50	5737	497	18	1536	—	1410	542	38	—	708	2512	5
27.403	78	4146	207	13	2875	—	—	73	64	1672	433	7321	5
1136.71	5.1	776	96	—	1212	—	950	1538	—	—	275	—	5
1136.14	5.3	4787	100	81	135	—	792	—	1400	—	7731	2408	5
1136.21	182	5844	391	506	9574	—	1357	—	65	—	—	—	4
27.384	46	—	281	357	—	—	—	—	65	—	458	—	4
1188.13	116	6923	58	382	—	—	1069	—	0.77	—	142	—	4
F107.10	120	2728	67	807	—	—	1647	—	5.5	—	135	—	4
F107.17	221	388	—	—	—	4878	5705	—	76	7440	299	3478	4
F107.23	163	5713	141	4113	—	—	6770	—	14	—	151	—	4
1136.12	105	720	129	14	2128	—	1503	—	343	2917	2500	—	4
1136.47	2.2	607	2119	303	755	—	5351	4255	—	—	—	—	4
1136.28	0.23	649	2623	2.2	1481	—	6627	9524	3182	7538	—	4478	3
1136.55	65	138	2451	—	—	—	271	6545	5000	—	—	—	3
1136.59.01a	130	39	—	—	29	—	3160	—	—	—	—	—	3
27.415	2011	754	718	653	—	—	—	4711	2234	8997	—	—	2
1136.46	68	985	5814	—	—	—	—	—	—	—	—	—	2

— Indicates binding affinity $\geq 10,000$ nM.

16 out of 29 (55%) degenerate on 5 or more DR types.

Table VI
 Degenerate "1-4-7" binders.

Degenerate "1-4-7" binders.

Binding Capacity (IC50% nM)

Peptide	Sequence	DRI-4,7	DRI	Other Alleles	Total Amino Acids									
1189.34	IIINWNIIVLAKLI	+	+		10									
1189.35	CLAYKVVVCAATY	+	+		9									
1189.36	KSKYKLVSLAGLL	+	+	-	+	+	+	+	+	+	+	+	+	9
1189.37	KYKLVSLAGLL	+	+	+	+	+	+	+	+	+	+	+	+	9
1189.38	KYKLVSLAGLL	+	+	+	+	+	+	+	+	+	+	+	+	9
1189.39	RIINWNIIVLAKLI	+	+	+	+	+	+	+	+	+	+	+	+	7
1189.40	AYKVVVCAATYAG	+	+	+	+	+	+	+	+	+	+	+	+	7
1189.41	VYFASFKLPIILA	+	+	+	+	+	+	+	+	+	+	+	+	7
1189.42	LTSQFLPALPFTWL	+	+	+	+	+	+	+	+	+	+	+	+	7
1189.43	IQEYKFAIVKPLA	+	+	+	+	+	+	+	+	+	+	+	+	7
1189.44	CPYALDSFGAIGTM	+	+	+	+	+	+	+	+	+	+	+	+	7
1189.45	SSVNVVNSIGLIM	+	+	+	+	+	+	+	+	+	+	+	+	6
1189.46	VKNVGFPAKVCVE	+	+	+	+	+	+	+	+	+	+	+	+	6
1189.47	LRVYVLSKAKCTV	+	+	+	+	+	+	+	+	+	+	+	+	6
1189.48	MRKLAIVSVSLFY	+	+	+	+	+	+	+	+	+	+	+	+	6
1189.49	SSIRCAFTSLI BCCC	+	+	+	+	+	+	+	+	+	+	+	+	5
1189.50	LVNLLRIIINCKIK	+	+	+	+	+	+	+	+	+	+	+	+	5
1189.51	EQQSTYAAASATSD	+	+	+	+	+	+	+	+	+	+	+	+	5
1189.52	FATCTPLTSLQFLP	+	+	+	+	+	+	+	+	+	+	+	+	5
1189.53	FNWVNSIGLIMVLS	+	+	+	+	+	+	+	+	+	+	+	+	5
1189.54	AGLGNVSTYLLCGV	+	+	+	+	+	+	+	+	+	+	+	+	4
1189.55	LAGLLGNVSTYLLCG	+	+	+	+	+	+	+	+	+	+	+	+	4
1189.56	TRHYPDLUGGAMLSL	+	+	+	+	+	+	+	+	+	+	+	+	4
1189.57	IKLPDLAFATCLIP	+	+	+	+	+	+	+	+	+	+	+	+	4
1189.58	VFNWVNSIGLIMVL	+	+	+	+	+	+	+	+	+	+	+	+	4
1189.59	NLSNVLATITTCVLI	+	+	+	+	+	+	+	+	+	+	+	+	4
1189.60	KFYVCAATPYAGER	+	+	+	+	+	+	+	+	+	+	+	+	3
1189.61	LAAILFLGPTTALDS	+	+	+	+	+	+	+	+	+	+	+	+	3
1189.62	QEDPLSTNTTYSNS	+	+	+	+	+	+	+	+	+	+	+	+	3
1189.63	RYVQERQVSTPQRAET	+	+	+	+	+	+	+	+	+	+	+	+	3
1189.64	NYKLVNVLIFPDL	+	+	+	+	+	+	+	+	+	+	+	+	2
1189.65	LWVSTMYLITRIITFDL	+	+	+	+	+	+	+	+	+	+	+	+	2
1189.66	WLPTRKFWVVTYASW	+	+	+	+	+	+	+	+	+	+	+	+	2

* Indicates binding affinity >1000nM.

Table VII

DR Type	Frequency of Binders	
	1-4-7 Degenerate Binders (%)	Non 1-4-7 Degenerate Binders (%)
1	31/32 (97)	17/67 (25)
4w4	22/32 (69)	8/67 (12)
7	21/32 (66)	7/67 (10)
9	20/32 (62)	2/67 (3.0)
6w19	18/32 (56)	6/67 (8.9)
2w28b	18/32 (56)	16/67 (24)
2w28a	16/32 (50)	10/67 (15)
4w15	12/32 (37)	4/67 (6.0)
8w2	10/32 (31)	3/67 (4.5)
5w11	9/32 (28)	6/67 (8.9)
5w12	3/32 (9.4)	4/67 (6.0)
3w17	1/32 (3.1)	0/67 (0)
w53	2/16 (13)	7/43 (16)

Table VIII, page 1

Protein	Sequence	Source	CR1	CR2	CR3	CR4	CR5	CR6	CR7	CR8	CR9	CR10	CR11	CR12	CR13	CR14	CR15	CR16	CR17	CR18	CR19	CR20	CR21	CR22	CR23	CR24	CR25	CR26	CR27	CR28	CR29	CR30	CR31	CR32	CR33	CR34	CR35	CR36	CR37	CR38	CR39	CR40	CR41	CR42	CR43	CR44	CR45	CR46	CR47	CR48	CR49	CR50	CR51	CR52	CR53	CR54	CR55	CR56	CR57	CR58	CR59	CR60	CR61	CR62	CR63	CR64	CR65	CR66	CR67	CR68	CR69	CR70	CR71	CR72	CR73	CR74	CR75	CR76	CR77	CR78	CR79	CR80	CR81	CR82	CR83	CR84	CR85	CR86	CR87	CR88	CR89	CR90	CR91	CR92	CR93	CR94	CR95	CR96	CR97	CR98	CR99	CR100	CR101	CR102	CR103	CR104	CR105	CR106	CR107	CR108	CR109	CR110	CR111	CR112	CR113	CR114	CR115	CR116	CR117	CR118	CR119	CR120	CR121	CR122	CR123	CR124	CR125	CR126	CR127	CR128	CR129	CR130	CR131	CR132	CR133	CR134	CR135	CR136	CR137	CR138	CR139	CR140	CR141	CR142	CR143	CR144	CR145	CR146	CR147	CR148	CR149	CR150	CR151	CR152	CR153	CR154	CR155	CR156	CR157	CR158	CR159	CR160	CR161	CR162	CR163	CR164	CR165	CR166	CR167	CR168	CR169	CR170	CR171	CR172	CR173	CR174	CR175	CR176	CR177	CR178	CR179	CR180	CR181	CR182	CR183	CR184	CR185	CR186	CR187	CR188	CR189	CR190	CR191	CR192	CR193	CR194	CR195	CR196	CR197	CR198	CR199	CR200	CR201	CR202	CR203	CR204	CR205	CR206	CR207	CR208	CR209	CR210	CR211	CR212	CR213	CR214	CR215	CR216	CR217	CR218	CR219	CR220	CR221	CR222	CR223	CR224	CR225	CR226	CR227	CR228	CR229	CR230	CR231	CR232	CR233	CR234	CR235	CR236	CR237	CR238	CR239	CR240	CR241	CR242	CR243	CR244	CR245	CR246	CR247	CR248	CR249	CR250	CR251	CR252	CR253	CR254	CR255	CR256	CR257	CR258	CR259	CR260	CR261	CR262	CR263	CR264	CR265	CR266	CR267	CR268	CR269	CR270	CR271	CR272	CR273	CR274	CR275	CR276	CR277	CR278	CR279	CR280	CR281	CR282	CR283	CR284	CR285	CR286	CR287	CR288	CR289	CR290	CR291	CR292	CR293	CR294	CR295	CR296	CR297	CR298	CR299	CR300	CR301	CR302	CR303	CR304	CR305	CR306	CR307	CR308	CR309	CR310	CR311	CR312	CR313	CR314	CR315	CR316	CR317	CR318	CR319	CR320	CR321	CR322	CR323	CR324	CR325	CR326	CR327	CR328	CR329	CR330	CR331	CR332	CR333	CR334	CR335	CR336	CR337	CR338	CR339	CR340	CR341	CR342	CR343	CR344	CR345	CR346	CR347	CR348	CR349	CR350	CR351	CR352	CR353	CR354	CR355	CR356	CR357	CR358	CR359	CR360	CR361	CR362	CR363	CR364	CR365	CR366	CR367	CR368	CR369	CR370	CR371	CR372	CR373	CR374	CR375	CR376	CR377	CR378	CR379	CR380	CR381	CR382	CR383	CR384	CR385	CR386	CR387	CR388	CR389	CR390	CR391	CR392	CR393	CR394	CR395	CR396	CR397	CR398	CR399	CR400	CR401	CR402	CR403	CR404	CR405	CR406	CR407	CR408	CR409	CR410	CR411	CR412	CR413	CR414	CR415	CR416	CR417	CR418	CR419	CR420	CR421	CR422	CR423	CR424	CR425	CR426	CR427	CR428	CR429	CR430	CR431	CR432	CR433	CR434	CR435	CR436	CR437	CR438	CR439	CR440	CR441	CR442	CR443	CR444	CR445	CR446	CR447	CR448	CR449	CR450	CR451	CR452	CR453	CR454	CR455	CR456	CR457	CR458	CR459	CR460	CR461	CR462	CR463	CR464	CR465	CR466	CR467	CR468	CR469	CR470	CR471	CR472	CR473	CR474	CR475	CR476	CR477	CR478	CR479	CR480	CR481	CR482	CR483	CR484	CR485	CR486	CR487	CR488	CR489	CR490	CR491	CR492	CR493	CR494	CR495	CR496	CR497	CR498	CR499	CR500	CR501	CR502	CR503	CR504	CR505	CR506	CR507	CR508	CR509	CR510	CR511	CR512	CR513	CR514	CR515	CR516	CR517	CR518	CR519	CR520	CR521	CR522	CR523	CR524	CR525	CR526	CR527	CR528	CR529	CR530	CR531	CR532	CR533	CR534	CR535	CR536	CR537	CR538	CR539	CR540	CR541	CR542	CR543	CR544	CR545	CR546	CR547	CR548	CR549	CR550	CR551	CR552	CR553	CR554	CR555	CR556	CR557	CR558	CR559	CR560	CR561	CR562	CR563	CR564	CR565	CR566	CR567	CR568	CR569	CR570	CR571	CR572	CR573	CR574	CR575	CR576	CR577	CR578	CR579	CR580	CR581	CR582	CR583	CR584	CR585	CR586	CR587	CR588	CR589	CR590	CR591	CR592	CR593	CR594	CR595	CR596	CR597	CR598	CR599	CR600	CR601	CR602	CR603	CR604	CR605	CR606	CR607	CR608	CR609	CR610	CR611	CR612	CR613	CR614	CR615	CR616	CR617	CR618	CR619	CR620	CR621	CR622	CR623	CR624	CR625	CR626	CR627	CR628	CR629	CR630	CR631	CR632	CR633	CR634	CR635	CR636	CR637	CR638	CR639	CR640	CR641	CR642	CR643	CR644	CR645	CR646	CR647	CR648	CR649	CR650	CR651	CR652	CR653	CR654	CR655	CR656	CR657	CR658	CR659	CR660	CR661	CR662	CR663	CR664	CR665	CR666	CR667	CR668	CR669	CR670	CR671	CR672	CR673	CR674	CR675	CR676	CR677	CR678	CR679	CR680	CR681	CR682	CR683	CR684	CR685	CR686	CR687	CR688	CR689	CR690	CR691	CR692	CR693	CR694	CR695	CR696	CR697	CR698	CR699	CR700	CR701	CR702	CR703	CR704	CR705	CR706	CR707	CR708	CR709	CR710	CR711	CR712	CR713	CR714	CR715	CR716	CR717	CR718	CR719	CR720	CR721	CR722	CR723	CR724	CR725	CR726	CR727	CR728	CR729	CR730	CR731	CR732	CR733	CR734	CR735	CR736	CR737	CR738	CR739	CR740	CR741	CR742	CR743	CR744	CR745	CR746	CR747	CR748	CR749	CR750	CR751	CR752	CR753	CR754	CR755	CR756	CR757	CR758	CR759	CR760	CR761	CR762	CR763	CR764	CR765	CR766	CR767	CR768	CR769	CR770	CR771	CR772	CR773	CR774	CR775	CR776	CR777	CR778	CR779	CR780	CR781	CR782	CR783	CR784	CR785	CR786	CR787	CR788	CR789	CR790	CR791	CR792	CR793	CR794	CR795	CR796	CR797	CR798	CR799	CR800	CR801	CR802	CR803	CR804	CR805	CR806	CR807	CR808	CR809	CR810	CR811	CR812	CR813	CR814	CR815	CR816	CR817	CR818	CR819	CR820	CR821	CR822	CR823	CR824	CR825	CR826	CR827	CR828	CR829	CR830	CR831	CR832	CR833	CR834	CR835	CR836	CR837	CR838	CR839	CR840	CR841	CR842	CR843	CR844	CR845	CR846	CR847	CR848	CR849	CR850	CR851	CR852	CR853	CR854	CR855	CR856	CR857	CR858	CR859	CR860	CR861	CR862	CR863	CR864	CR865	CR866	CR867	CR868	CR869	CR870	CR871	CR872	CR873	CR874	CR875	CR876	CR877	CR878	CR879	CR880	CR881	CR882	CR883	CR884	CR885	CR886	CR887	CR888	CR889	CR890	CR891	CR892	CR893	CR894	CR895	CR896	CR897	CR898	CR899	CR900	CR901	CR902	CR903	CR904	CR905	CR906	CR907	CR908	CR909	CR910	CR911	CR912	CR913	CR914	CR915	CR916	CR917	CR918	CR919	CR920	CR921	CR922	CR923	CR924	CR925	CR926	CR927	CR928	CR929	CR930	CR931	CR932	CR933	CR934	CR935	CR936	CR937	CR938	CR939	CR940	CR941	CR942	CR943	CR944	CR945	CR946	CR947	CR948	CR949	CR950	CR951	CR952	CR953	CR954	CR955	CR956	CR957	CR958	CR959	CR960	CR961	CR962	CR963	CR964	CR965	CR966	CR967	CR968	CR969	CR970	CR971	CR972	CR973	CR974	CR975	CR976	CR977	CR978	CR979	CR980	CR981	CR982	CR983	CR984	CR985	CR986	CR987	CR988	CR989	CR990	CR991	CR992	CR993	CR994	CR995	CR996	CR997	CR998	CR999	CR1000	CR1001	CR1002	CR1003	CR1004	CR1005	CR1006	CR1007	CR1008	CR1009	CR1010	CR1011	CR1012	CR1013	CR1014	CR1015	CR1016	CR1017	CR1018	CR1019	CR1020	CR1021	CR1022	CR1023	CR1024	CR1025	CR1026	CR1027	CR1028	CR1029	CR1030	CR1031	CR1032	CR1033	CR1034	CR1035	CR1036	CR1037	CR1038	CR1039	CR1040	CR1041	CR1042	CR1043	CR1044	CR1045	CR1046	CR1047	CR1048	CR1049	CR1050	CR1051	CR1052	CR1053	CR1054	CR1055	CR1056	CR1057	CR1058	CR1059	CR1060	CR1061	CR1062	CR1063	CR1064	CR1065	CR1066	CR1067	CR1068	CR1069	CR1070	CR1071	CR1072	CR1073	CR1074	CR1075	CR1076	CR1077	CR1078	CR1079	CR1080	CR1081	CR1082	CR1083	CR1084	CR1085	CR1086	CR1087	CR1088	CR1089	CR1090	CR1091	CR1092	CR1093	CR1094	CR1095	CR1096	CR1097	CR1098	CR1099	CR1100	CR1101	CR1102	CR1103	CR1104	CR1105	CR1106	CR1107	CR1108	CR1109	CR1110	CR1111	CR1112	CR1113	CR1114	CR1115	CR1116	CR1117	CR1118	CR1119	CR1120	CR1121	CR1122	CR1123	CR1124	CR1125	CR1126	CR1127	CR1128	CR1129	CR1130	CR1131	CR1132	CR1133	CR1134	CR1135	CR1136	CR1137	CR1138	CR1139	CR1140	CR1141	CR1142	CR1143	CR1144	CR1145	CR1146	CR1147	CR1148	CR1149	CR1150	CR1151	CR1152	CR1153	CR1154	CR1155	CR1156	CR1157	CR1158	CR1159	CR1160	CR1161	CR1162	CR1163	CR1164	CR1165	CR1166	CR1167	CR1168	CR1169	CR1170	CR1171	CR1172	CR1173	CR1174	CR1175	CR1176	CR1177	CR1178	CR1179	CR1180	CR1181	CR1182	CR1183	CR1184	CR1185	CR1186	CR1187	CR1188	CR1189	CR1190	CR1191	CR1192	CR1193	CR1194	CR1195	CR1196	CR1197	CR1198	CR1199	CR1200	CR1201	CR1202	CR1203	CR1204	CR1205	CR1206	CR1207	CR1208	CR1209	CR1210	CR1211	CR1212	CR1213	CR1214	CR1215	CR1216	CR1217	CR1218	CR1219	CR1220	CR1221	CR1222	CR1223	CR1224	CR1225	CR1226	CR1227	CR1228	CR1229	CR1230	CR1231	CR1232	CR1233	CR1234	CR1235	CR1236	CR1237	CR1238	CR1239	CR1240	CR1241	CR1242	CR1243	CR1244	CR1245	CR1246	CR1247	CR1248	CR1249	CR1250	CR1251	CR1252	CR1253	CR1254	CR1255	CR1256	CR1257	CR1258	CR1259	CR1260	CR1261	CR1262	CR1263	CR1264	CR1265	CR1266	CR1267	CR1268	CR1269	CR1270	CR1271	CR1272	CR1273	CR1274	CR1275	CR1276	CR1277	CR1278	CR1279	CR1280	CR1281	CR1282	CR1283	CR1284	CR1285	CR1286	CR1287	CR1288	CR1289	CR1290	CR1291	CR1292	CR1293	CR1294	CR1295	CR1296	CR1297	CR1298	CR1299	CR1300	CR1301	CR1302	CR1303	CR1304	CR1305	CR1306	CR1307	CR1308	CR1309	CR1310	CR1311	CR1312	CR1313	CR1314	CR1315	CR1316	CR1317	CR1318	CR1319	CR1320	CR1321	CR1322	CR1323	CR1324	CR1325	CR1326	CR1327	CR1328	CR
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Table VIII, page 2

Page	Sequence	Source	DET	DET-381	DET-312	DET-4	DET-16	DET-11	DET-12	DET-19	DET	DET-2	DET-3	DET-23
38.0275	GERLIPACTIV	MedImm.838	(10000.0)	168.5	7467.4	(10000.0)	407.8	2002.5	(11000.7)	200.0	(22727.3)	9007.8		
38.0276	OFFCTIVYAK	MedImm.843	74.6	1230.7	4761.6	7500.0	500.1			182.3	(22727.3)	1048.9		
38.0277	OFFCTIVYAK	MedImm.848	(10000.0)			1001.5					(22727.3)			
38.0278	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0279	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0280	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0281	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0282	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0283	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0284	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0285	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0286	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0287	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0288	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0289	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0290	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0291	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0292	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0293	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0294	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0295	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0296	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0297	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0298	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0299	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0300	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0301	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0302	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0303	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0304	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0305	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0306	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0307	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0308	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0309	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0310	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0311	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0312	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0313	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0314	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0315	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0316	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0317	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0318	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0319	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0320	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0321	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0322	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0323	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0324	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0325	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0326	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0327	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0328	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0329	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0330	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0331	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0332	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0333	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0334	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			
38.0335	OFFCTIVYAK	MedImm.1028	(10000.0)			1001.5					(22727.3)			

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Page No	Sequence	Source	DRI	ORIG-201	ORIG-102	ORIG-1	ORIG-12	ORIG-18	ORIG-2	ORIG-3
			ml	ml	ml	ml	ml	ml	ml	ml
32,2967	NOVAJANUCORIN	MA022294			(120000.0)					
32,2968	MA022295	(115000.0)								
32,2969	MA022296	(115000.0)								
32,2970	MA022297	(115000.0)								
32,2971	MA022298	(115000.0)								
32,2972	MA022299	(115000.0)								
32,2973	MA022300	(115000.0)								
32,2974	MA022301	(115000.0)								
32,2975	MA022302	(115000.0)								
32,2976	MA022303	(115000.0)								
32,2977	MA022304	(115000.0)								
32,2978	MA022305	(115000.0)								
32,2979	MA022306	(115000.0)								
32,2980	MA022307	(115000.0)								
32,2981	MA022308	(115000.0)								
32,2982	MA022309	(115000.0)								
32,2983	MA022310	(115000.0)								
32,2984	MA022311	(115000.0)								
32,2985	MA022312	(115000.0)								
32,2986	MA022313	(115000.0)								
32,2987	MA022314	(115000.0)								
32,2988	MA022315	(115000.0)								
32,2989	MA022316	(115000.0)								
32,2990	MA022317	(115000.0)								
32,2991	MA022318	(115000.0)								
32,2992	MA022319	(115000.0)								
32,2993	MA022320	(115000.0)								
32,2994	MA022321	(115000.0)								
32,2995	MA022322	(115000.0)								
32,2996	MA022323	(115000.0)								
32,2997	MA022324	(115000.0)								
32,2998	MA022325	(115000.0)								
32,2999	MA022326	(115000.0)								
32,3000	MA022327	(115000.0)								
32,3001	MA022328	(115000.0)								
32,3002	MA022329	(115000.0)								
32,3003	MA022330	(115000.0)								
32,3004	MA022331	(115000.0)								
32,3005	MA022332	(115000.0)								
32,3006	MA022333	(115000.0)								
32,3007	MA022334	(115000.0)								
32,3008	MA022335	(115000.0)								
32,3009	MA022336	(115000.0)								
32,3010	MA022337	(115000.0)								
32,3011	MA022338	(115000.0)								
32,3012	MA022339	(115000.0)								
32,3013	MA022340	(115000.0)								
32,3014	MA022341	(115000.0)								
32,3015	MA022342	(115000.0)								
32,3016	MA022343	(115000.0)								
32,3017	MA022344	(115000.0)								
32,3018	MA022345	(115000.0)								
32,3019	MA022346	(115000.0)								
32,3020	MA022347	(115000.0)								
32,3021	MA022348	(115000.0)								
32,3022	MA022349	(115000.0)								
32,3023	MA022350	(115000.0)								
32,3024	MA022351	(115000.0)								
32,3025	MA022352	(115000.0)								
32,3026	MA022353	(115000.0)								
32,3027	MA022354	(115000.0)								
32,3028	MA022355	(115000.0)								
32,3029	MA022356	(115000.0)								
32,3030	MA022357	(115000.0)								
32,3031	MA022358	(115000.0)								
32,3032	MA022359	(115000.0)								
32,3033	MA022360	(115000.0)								
32,3034	MA022361	(115000.0)								
32,3035	MA022362	(115000.0)								
32,3036	MA022363	(115000.0)								
32,3037	MA022364	(115000.0)								
32,3038	MA022365	(115000.0)								
32,3039	MA022366	(115000.0)								
32,3040	MA022367	(115000.0)								
32,3041	MA022368	(115000.0)								
32,3042	MA022369	(115000.0)								
32,3043	MA022370	(115000.0)								
32,3044	MA022371	(115000.0)								
32,3045	MA022372	(115000.0)								
32,3046	MA022373	(115000.0)								
32,3047	MA022374	(115000.0)								
32,3048	MA022375	(115000.0)								
32,3049	MA022376	(115000.0)								
32,3050	MA022377	(115000.0)								
32,3051	MA022378	(115000.0)								
32,3052	MA022379	(115000.0)								
32,3053	MA022380	(115000.0)								
32,3054	MA022381	(115000.0)								
32,3055	MA022382	(115000.0)								
32,3056	MA022383	(115000.0)								
32,3057	MA022384	(115000.0)								
32,3058	MA022385	(115000.0)								
32,3059	MA022386	(115000.0)								
32,3060	MA022387	(115000.0)								
32,3061	MA022388	(115000.0)								
32,3062	MA022389	(115000.0)								
32,3063	MA022390	(115000.0)								
32,3064	MA022391	(115000.0)								
32,3065	MA022392	(115000.0)								
32,3066	MA022393	(115000.0)								
32,3067	MA022394	(115000.0)								
32,3068	MA022395	(115000.0)								
32,3069	MA022396	(115000.0)								
32,3070	MA022397	(115000.0)								
32,3071	MA022398	(115000.0)								
32,3072	MA022399	(115000.0)								
32,3073	MA022400	(115000.0)								
32,3074	MA022401	(115000.0)								
32,3075	MA022402	(115000.0)								
32,3076	MA022403	(115000.0)								
32,3077	MA022404	(115000.0)								
32,3078	MA022405	(115000.0)								
32,3079	MA022406	(115000.0)								
32,3080	MA022407	(115000.0)								
32,3081	MA022408	(115000.0)								
32,3082	MA022409	(115000.0)								
32,3083	MA022410	(115000.0)								
32,3084	MA022411	(115000.0)								
32,3085	MA022412	(115000.0)								
32,3086	MA022413	(115000.0)								
32,3087	MA022414	(115000.0)								
32,3088	MA022415	(115000.0)								
32,3089	MA022416	(115000.0)								
32,3090	MA022417	(115000.0)								
32,3091	MA022418	(115000.0)								
32,3092	MA022419	(115000.0)								
32,3093	MA022420	(115000.0)								
32,3094	MA022421	(115000.0)								
32,3095	MA022422	(115000.0)								
32,3096	MA022423	(115000.0)								
32,3097	MA022424	(115000.0)								
32,3098	MA022425	(115000.0)								
32,3099	MA022426	(115000.0)								
32,3100	MA022427	(115000.0)								
32,3101	MA022428	(115000.0)								
32,3102	MA022429	(115000.0)								
32,3103	MA022430	(115000.0)								
32,3104	MA022431	(115000.0)								
32,3105	MA022432	(115000.0)								
32,3106	MA022433	(115000.0)								
32,3107	MA022434	(115000.0)								
32,3108	MA022435	(115000.0)								
32,3109	MA022436	(115000.0)								
32,3110	MA022437	(115000.0)								
32,3111	MA022438	(115000.0)								
32,3112	MA022439	(115000.0)								
32,3113	MA022440	(115000.0)								
32,3114	MA022441	(115000.0)								
32,3115	MA022442	(115000.0)								
32,3116	MA022443	(115000.0)								
32,3117	MA022444	(115000.0)								
32,3118	MA022445	(115000.0)								
32,3119	MA022446	(115000.0)								
32,3120	MA022447	(115000.0)								
32,3121	MA022448	(115000.0)								
32,3122	MA022449	(115000.0)								
32,3123	MA022450	(115000.0)								
32,3124	MA022451	(115000.0)								
32,3125	MA022452	(115000.0)								
32,3126	MA022453	(115000.0)								
32,3127	MA022454	(115000.0)								
32,3128	MA022455	(115000.0)								
32,3129	MA022456	(115000.0)								
32,3130	MA022457	(115000.0)								
32,3131	MA022458	(115000.0)								
32,3132	MA022459	(115000.0)								
32,3133	MA022460	(115000.0)								
32,3134	MA0224									

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Payee	Sequence	Source	DT1	DT2	DT3	DT4	DT5	DT6	DT7	DT8	DT9	DT10	DT11	DT12	DT13	DT14	DT15	DT16	DT17	DT18	DT19	DT20	DT21	DT22	DT23	DT24	DT25	DT26	DT27	DT28	DT29	DT30	DT31	DT32	DT33	DT34	DT35	DT36	DT37	DT38	DT39	DT40	DT41	DT42	DT43	DT44	DT45	DT46	DT47	DT48	DT49	DT50	DT51	DT52	DT53	DT54	DT55	DT56	DT57	DT58	DT59	DT60	DT61	DT62	DT63	DT64	DT65	DT66	DT67	DT68	DT69	DT70	DT71	DT72	DT73	DT74	DT75	DT76	DT77	DT78	DT79	DT80	DT81	DT82	DT83	DT84	DT85	DT86	DT87	DT88	DT89	DT90	DT91	DT92	DT93	DT94	DT95	DT96	DT97	DT98	DT99	DT100	DT101	DT102	DT103	DT104	DT105	DT106	DT107	DT108	DT109	DT110	DT111	DT112	DT113	DT114	DT115	DT116	DT117	DT118	DT119	DT120	DT121	DT122	DT123	DT124	DT125	DT126	DT127	DT128	DT129	DT130	DT131	DT132	DT133	DT134	DT135	DT136	DT137	DT138	DT139	DT140	DT141	DT142	DT143	DT144	DT145	DT146	DT147	DT148	DT149	DT150	DT151	DT152	DT153	DT154	DT155	DT156	DT157	DT158	DT159	DT160	DT161	DT162	DT163	DT164	DT165	DT166	DT167	DT168	DT169	DT170	DT171	DT172	DT173	DT174	DT175	DT176	DT177	DT178	DT179	DT180	DT181	DT182	DT183	DT184	DT185	DT186	DT187	DT188	DT189	DT190	DT191	DT192	DT193	DT194	DT195	DT196	DT197	DT198	DT199	DT200	DT201	DT202	DT203	DT204	DT205	DT206	DT207	DT208	DT209	DT210	DT211	DT212	DT213	DT214	DT215	DT216	DT217	DT218	DT219	DT220	DT221	DT222	DT223	DT224	DT225	DT226	DT227	DT228	DT229	DT230	DT231	DT232	DT233	DT234	DT235	DT236	DT237	DT238	DT239	DT240	DT241	DT242	DT243	DT244	DT245	DT246	DT247	DT248	DT249	DT250	DT251	DT252	DT253	DT254	DT255	DT256	DT257	DT258	DT259	DT260	DT261	DT262	DT263	DT264	DT265	DT266	DT267	DT268	DT269	DT270	DT271	DT272	DT273	DT274	DT275	DT276	DT277	DT278	DT279	DT280	DT281	DT282	DT283	DT284	DT285	DT286	DT287	DT288	DT289	DT290	DT291	DT292	DT293	DT294	DT295	DT296	DT297	DT298	DT299	DT300	DT301	DT302	DT303	DT304	DT305	DT306	DT307	DT308	DT309	DT310	DT311	DT312	DT313	DT314	DT315	DT316	DT317	DT318	DT319	DT320	DT321	DT322	DT323	DT324	DT325	DT326	DT327	DT328	DT329	DT330	DT331	DT332	DT333	DT334	DT335	DT336	DT337	DT338	DT339	DT340	DT341	DT342	DT343	DT344	DT345	DT346	DT347	DT348	DT349	DT350	DT351	DT352	DT353	DT354	DT355	DT356	DT357	DT358	DT359	DT360	DT361	DT362	DT363	DT364	DT365	DT366	DT367	DT368	DT369	DT370	DT371	DT372	DT373	DT374	DT375	DT376	DT377	DT378	DT379	DT380	DT381	DT382	DT383	DT384	DT385	DT386	DT387	DT388	DT389	DT390	DT391	DT392	DT393	DT394	DT395	DT396	DT397	DT398	DT399	DT400	DT401	DT402	DT403	DT404	DT405	DT406	DT407	DT408	DT409	DT410	DT411	DT412	DT413	DT414	DT415	DT416	DT417	DT418	DT419	DT420	DT421	DT422	DT423	DT424	DT425	DT426	DT427	DT428	DT429	DT430	DT431	DT432	DT433	DT434	DT435	DT436	DT437	DT438	DT439	DT440	DT441	DT442	DT443	DT444	DT445	DT446	DT447	DT448	DT449	DT450	DT451	DT452	DT453	DT454	DT455	DT456	DT457	DT458	DT459	DT460	DT461	DT462	DT463	DT464	DT465	DT466	DT467	DT468	DT469	DT470	DT471	DT472	DT473	DT474	DT475	DT476	DT477	DT478	DT479	DT480	DT481	DT482	DT483	DT484	DT485	DT486	DT487	DT488	DT489	DT490	DT491	DT492	DT493	DT494	DT495	DT496	DT497	DT498	DT499	DT500	DT501	DT502	DT503	DT504	DT505	DT506	DT507	DT508	DT509	DT510	DT511	DT512	DT513	DT514	DT515	DT516	DT517	DT518	DT519	DT520	DT521	DT522	DT523	DT524	DT525	DT526	DT527	DT528	DT529	DT530	DT531	DT532	DT533	DT534	DT535	DT536	DT537	DT538	DT539	DT540	DT541	DT542	DT543	DT544	DT545	DT546	DT547	DT548	DT549	DT550	DT551	DT552	DT553	DT554	DT555	DT556	DT557	DT558	DT559	DT560	DT561	DT562	DT563	DT564	DT565	DT566	DT567	DT568	DT569	DT570	DT571	DT572	DT573	DT574	DT575	DT576	DT577	DT578	DT579	DT580	DT581	DT582	DT583	DT584	DT585	DT586	DT587	DT588	DT589	DT590	DT591	DT592	DT593	DT594	DT595	DT596	DT597	DT598	DT599	DT600	DT601	DT602	DT603	DT604	DT605	DT606	DT607	DT608	DT609	DT610	DT611	DT612	DT613	DT614	DT615	DT616	DT617	DT618	DT619	DT620	DT621	DT622	DT623	DT624	DT625	DT626	DT627	DT628	DT629	DT630	DT631	DT632	DT633	DT634	DT635	DT636	DT637	DT638	DT639	DT640	DT641	DT642	DT643	DT644	DT645	DT646	DT647	DT648	DT649	DT650	DT651	DT652	DT653	DT654	DT655	DT656	DT657	DT658	DT659	DT660	DT661	DT662	DT663	DT664	DT665	DT666	DT667	DT668	DT669	DT670	DT671	DT672	DT673	DT674	DT675	DT676	DT677	DT678	DT679	DT680	DT681	DT682	DT683	DT684	DT685	DT686	DT687	DT688	DT689	DT690	DT691	DT692	DT693	DT694	DT695	DT696	DT697	DT698	DT699	DT700	DT701	DT702	DT703	DT704	DT705	DT706	DT707	DT708	DT709	DT710	DT711	DT712	DT713	DT714	DT715	DT716	DT717	DT718	DT719	DT720	DT721	DT722	DT723	DT724	DT725	DT726	DT727	DT728	DT729	DT730	DT731	DT732	DT733	DT734	DT735	DT736	DT737	DT738	DT739	DT740	DT741	DT742	DT743	DT744	DT745	DT746	DT747	DT748	DT749	DT750	DT751	DT752	DT753	DT754	DT755	DT756	DT757	DT758	DT759	DT760	DT761	DT762	DT763	DT764	DT765	DT766	DT767	DT768	DT769	DT770	DT771	DT772	DT773	DT774	DT775	DT776	DT777	DT778	DT779	DT780	DT781	DT782	DT783	DT784	DT785	DT786	DT787	DT788	DT789	DT790	DT791	DT792	DT793	DT794	DT795	DT796	DT797	DT798	DT799	DT800	DT801	DT802	DT803	DT804	DT805	DT806	DT807	DT808	DT809	DT810	DT811	DT812	DT813	DT814	DT815	DT816	DT817	DT818	DT819	DT820	DT821	DT822	DT823	DT824	DT825	DT826	DT827	DT828	DT829	DT830	DT831	DT832	DT833	DT834	DT835	DT836	DT837	DT838	DT839	DT840	DT841	DT842	DT843	DT844	DT845	DT846	DT847	DT84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Page	Sequence	Source	CON	DATE/201	INFO-002	DPT	DATE-1	In ICED Format	DATE-11	DATE-12	DATE-13	DPT	DATE-14	DPT	DATE-15
011.01	PPHCHCHILAT	HA 207-310 ending	6.9		24.5		100.0	500000.0	1000.0				304.9		00000.0
011.02	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		35.0	1210.0	210.0				100.0		00000.0
011.03	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		75.0	5150.0	1233.3				100.0		00000.0
011.04	PPHCHCHILAT	HA 207-310 ending	7.4		25.5		25.4	210.0	10.0				100.0		00000.0
011.05	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		137.0	21000.0	200.0				100.0		00000.0
011.06	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.07	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.08	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.09	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.10	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.11	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.12	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.13	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.14	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.15	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.16	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.17	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.18	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.19	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.20	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.21	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.22	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.23	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.24	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.25	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.26	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.27	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.28	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.29	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.30	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.31	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.32	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.33	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.34	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.35	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.36	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.37	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.38	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.39	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.40	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.41	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.42	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.43	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.44	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.45	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.46	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.47	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.48	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.49	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.50	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.51	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.52	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.53	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.54	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.55	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.56	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.57	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.58	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.59	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.60	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.61	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.62	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.63	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.64	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.65	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.66	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.67	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.68	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.69	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.70	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.71	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.72	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.73	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.74	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.75	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.76	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.77	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.78	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.79	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.80	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.81	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.82	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.83	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.84	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.85	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.86	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.87	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.88	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.89	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.90	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.91	PPHCHCHILAT	HA 207-310 ending	5.9		25.5		25.7	1111.1	100.0				100.0		00000.0
011.92	PPHCHCHILAT	HA 207-310 ending	5.9		25.										

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TABLE IX

MOTIFS	POSITION					
	1° anchor 1	2	3	4	5	6
DR4 preferred deleterious	FMYLIVW	M	T	W	I	VSTCPALIM MH R
DR1 preferred deleterious	MFLIVWY			PAMQ FD	CWD	VMATSP LIC M GDE D
DR7 preferred deleterious	MFLIVWY	M C	W C	A G		IVMSACTPL M GRD N
DR Supermotif	MFLIVWY					VMSTACPLI
DR3 MOTIFS	1° anchor 1	2	3	1° anchor 4	5	1° anchor 6
motif a preferred	LIVMFY			D		
motif b preferred	LIVMFAY			DNQEST		KRH

Italicized residues indicate less preferred or "tolerated" residues.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a unit dose form of a peptide comprising an epitope from Table VIII or analog thereof which binds to an HLA class II molecule at an IC_{50} of less than or equal to 1,000 nM.
2. The composition of claim 1, wherein the peptide is derived from a tumor antigen which is selected from the group consisting of carcinoembryonic antigen (CEA), p53, MAGE-2, MAGE-3, or Her2/neu.
3. The composition of claim 1, wherein the immunogenic peptide is derived from a viral antigen.
4. The composition of claim 3, wherein the viral antigen is from HIV, HBV, or HCV.
5. The composition of claim 5, wherein the antigen is *Plasmodium falciparum*.
6. A composition of claim 1 wherein the epitope comprises an amino acid that is Y, F, W, L, I, V, or M at the first position from the N-terminus of the epitope and an amino acid of S, T, C, A, P, V, I, L, or M at the sixth position from the N-terminus of the epitope.
7. A composition of claim 1 wherein the composition is a nucleic acid that encodes the peptide.
8. A method of inducing a helper T cell response in a patient, the method comprising contacting a helper T cell with a composition of claim 1.
9. The method of claim 9, wherein the composition is a nucleic acid that encodes the peptide.

10. A composition comprising an epitope from Table VIII or analog thereof which binds to an HLA class II molecule at an IC_{50} of less than or equal to 1,000 nM wherein the epitope is bound to an HLA class II molecule present on an antigen presenting cell.
11. A composition that comprises at least two peptides of claim 1.
12. A composition that comprises at least three peptides of claim 1.
13. A composition of claim 1, wherein a unit dose form of the peptide is in the range of between 500 μ g and 50,000 μ g.

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RESIDUE	p1 ANCHOR	2	3	4	5	p6 ANCHOR	7	8	9
C		0.57	0.74	1.12	0.83	0.47	0.94	0.28	1.10
G		1.14	0.64	0.43	0.48		0.49	1.19	0.52
S		1.55	1.31	1.29	1.76	1.11	1.23	2.93	1.54
T		1.00	4.34	0.89	1.32	1.86	3.07	1.76	1.64
P		0.56	0.31	1.44	2.46	0.86	2.83	2.12	2.18
A		0.96	1.04	1.57	0.59	0.65	0.86	0.82	1.62
L	0.81	0.86	1.88	1.28	1.11	0.67	1.36	1.08	0.83
I	0.79	1.74	1.01	1.91	4.39	0.98	2.36	1.66	2.75
V	0.79	3.34	0.93	1.05	0.70	2.36	0.69	0.54	1.53
M	1.14	12.79	1.49	2.77	0.32	0.74	8.11	1.98	4.05
F	2.33	3.66	1.85	0.80	1.58		1.84	1.34	1.12
W	0.82	2.04	2.52	0.21	0.91		0.39	0.35	0.22
Y	1.07	0.74	1.51	0.39	1.41		0.44	0.61	0.35
H		0.78	0.15	1.14	0.93		13.77	1.40	5.15
R		1.09	0.50	0.69	0.39		0.14	0.41	1.22
K		1.44	1.25	0.53	0.40		0.62	0.64	0.55
Q		0.40	0.38	1.61	2.09		0.31	0.71	0.62
N		0.44	1.72	1.42	1.89		0.84	0.43	1.64
D		0.34	0.33	1.40	0.40		0.58	0.53	0.24
E		0.31	1.09	0.42	0.42		0.29	0.61	0.25

FIG. 1.

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RESIDUE	p1 ANCHOR	2	3	4	5	p6 ANCHOR	7	8	9
C		0.22	0.15	0.49	0.06	0.14	0.31	0.45	0.35
G		1.29	3.38	2.13	1.73		0.23	1.58	0.44
S		0.87	0.48	0.32	0.58	0.74	1.03	1.25	1.03
T		0.57	2.08	0.30	1.59	1.26	1.51	1.73	2.32
P		0.43	0.88	5.42	2.57	0.63	1.78	1.63	1.52
A		1.93	3.51	4.14	1.59	2.42	1.89	1.25	4.09
L	0.97	1.20	0.64	3.08	2.32	0.85	2.02	3.10	0.83
I	1.00	3.84	1.59	1.10	1.30	0.75	3.47	0.67	1.32
V	0.74	2.95	1.08	0.79	1.97	1.16	2.89	0.57	5.89
M	2.82	1.07	2.62	7.66	0.93	2.67	7.27	1.01	4.39
F	1.51	2.05	0.49	0.22	0.40		0.91	0.89	0.79
W	0.30	0.63	0.69	0.56	0.14		0.61	0.35	0.58
Y	0.88	0.51	1.22	0.36	2.04		0.99	0.26	0.42
H		0.51	0.11	0.68	1.57		1.81	1.20	0.55
R		0.80	0.49	0.43	0.37		1.08	1.43	0.83
K		2.69	2.32	0.49	0.67		1.33	2.24	0.44
Q		1.38	1.27	7.07	1.58		1.06	3.65	1.54
N		0.63	1.41	1.20	0.75		1.16	0.43	1.15
D		0.85	0.31	0.20	0.21		0.11	0.08	0.39
E		0.31	0.47	0.59	0.57		0.16	0.53	0.27

FIG. 2.

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RESIDUE	p1 ANCHOR	2	3	4	5	p6 ANCHOR	7	8	9
C		0.17	0.58	0.30	0.26	0.45	1.38	0.53	1.04
G		0.45	0.43	0.25	0.54		0.23	1.30	0.22
S		1.86	0.66	1.11	2.39	1.14	1.95	1.67	0.89
T		0.72	6.53	1.88	1.78	0.79	1.54	0.94	1.92
P		0.36	0.37	2.01	0.46	0.49	1.06	0.60	1.78
A		1.43	2.63	4.78	0.89	1.51	0.74	0.89	0.61
L	0.87	1.04	1.08	1.09	0.83	0.89	1.88	1.18	0.97
I	0.77	1.99	0.96	2.17	2.88	1.11	1.11	1.52	5.69
V	0.82	2.15	0.47	0.57	0.92	2.25	1.36	0.80	5.49
M	1.45	5.75	2.54	3.74	0.33	1.21	9.03	3.01	3.42
F	1.97	1.43	0.68	0.90	1.07		2.50	2.39	1.90
W	0.93	1.32	4.07	0.81	0.58		0.81	0.95	0.66
Y	0.90	0.78	3.34	0.62	3.32		0.64	0.74	0.74
H		1.67	0.36	0.62	2.09		1.10	1.02	1.13
R		1.29	0.70	0.45	1.31		0.21	0.59	2.67
K		1.45	1.32	0.47	0.86		1.40	1.26	0.48
Q		1.70	0.82	2.09	1.4		1.01	2.68	0.36
N		1.42	2.35	0.86	1.68		1.62	0.24	0.88
D		0.61	0.41	0.27	0.26		0.19	0.44	0.30
E		0.48	0.59	1.23	0.74		0.45	0.57	1.16

FIG. 3.

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12066

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.23, 7.24, 343.2, 344; 424/160.1, 159.1, 174.1, 188.1, 189.1, 208.1, 227.1; 530/388.35, 388.3, 388.75, 388.8, 389.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG, BIOSIS, DISSERTATION ABSTRACTS ONLINE, EMBASE, MEDLINE, AIDSLINE, epitope, carcinoembryonic antigen, HLA, MAGE, HIV, CLASS II, tumor antigen

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	VALMORI ET AL. Analysis of MAGE-3-specific Cytolytic T Lymphocytes in Human Leukocyte Antigen-A2 Melanoma Patients. Cancer Research. 15 February 1997. Vol 57. No. 4. pages 735-741, especially Abstract.	1-13
Y	HARRISON ET AL. A Peptide-binding Motif for I-Ag7, the Class II Major Histocompatibility Complex (MHC) Molecule of NOD and Biozzi AB/H Mice. J. Exp. Med. 17 March 1997. Vol 185. No. 6. pages 1013-1021, especially Abstract.	1-13

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T*
A document defining the general state of the art which is not considered to be of particular relevance	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
B earlier document published on or after the international filing date	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O document referring to an oral disclosure, use, exhibition or other means	*A* document member of the same patent family
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

16 AUGUST 1999

Date of mailing of the international search report

21 OCT 1999

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12066

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FRAZIANO ET AL. The Presence of Antibodies against HIV Peptides in the Sera of Alloimmune Mice and Thalassemic Patients Is Due to a Polyclonal Activation mechanism. Clinical Immunology and Immunopathology. August 1997. Vol 84. No. 2. pages 202-207, especially Abstract.	1-13
Y	BREMERS, ET AL. The Use of Epstein-Barr Virus-Transformed B Lymphocyte Cell Lines in a Peptide-Reconstitution Assay: Identification of CEA-Related HLA-A *0301-Restricted potential Cytotoxic T-Lymphocyte Epitopes. J. Immunotherapy. August 1995. Vol 18. No. 2. pages 77-85, especially Abstract.	1-13
Y	ZAREMBA ET AL. Identification of an Enhancer Agonist Cytotoxic T lymphocyte Peptide from Human Carcinoembryonic Antigen. Cancer Research. 15 October 1997. Vol 57. pages 4570-4577, especially Abstract.	1-13
Y	RAS ET AL. Identification of Potential HLA-A *0201 Restricted CTL Epitopes Derived from the Epithelial Cell Adhesion Molecule (Ep-CAM) and the Carcinoembryonic Antigen (CEA). Human Immunology. January 1997. Vol 53. pages 81-89, especially Abstract.	1-13

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12066

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

G01N 33/574, 33/53; C12N 7/00; A61K 39/42, 39/395, 39/21, 39/29; C07K 16/00

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/7.23, 7.24, 343.2, 344; 424/160.1, 159.1, 174.1, 188.1, 189.1, 208.1, 227.1; 530/388.35, 388.3, 388.75, 388.8, 389.4

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